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CORTICAL NECROSIS OF THE ADRENAL GLANDS ASSOCIATED WITH ADDISON'S DISEASE

REPORT OF EIGHT CASES

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Addison's disease in the majority of cases is due to tuberculous destruction of the adrenal glands. In Addison's series of 11 cases [1] there was 1 in which "the two supra-renal capsules . . . appeared exceedingly small and atrophied." The nontuberculous nature of the disease in such cases was later established, and the condition of the adrenal glands has subsequently been described under many names, of which the most popular in the English literature has been "adrenal atrophy." Until recently the frequency of "adrenal atrophy" as a cause of Addison's disease has been generally estimated to be about 10 per cent on this continent and somewhat higher in Europe.

In the department of pathology of the University of Toronto interest in this subject was recently stimulated by the study of 3 cases. A survey of the autopsy records of the Toronto General Hospital from the beginning of 1924 until the time of writing revealed 17 cases of Addison's disease in a series of some 7,000 autopsies on adults. In 10 of these the condition was due to tuberculosis, and in 7 (41 per cent) to "atrophy," of the adrenal glands. Although this series is not large, the figures are in agreement with those of several reports published during the past decade,[2] which indicate an incidence of "atrophy" as a cause of Addison's disease much higher than 10 per cent.

Relatively few cases of Addison's disease associated with the type of lesion under consideration have been recorded in detail. For this reason and in an effort to demonstrate that the process is one of cortical

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1. Addison, T.: On the Constitutional and Local Effects of Disease of the Supra-Renal Capsules, London, S. Highley, 1855; M. Classics **2**:239, 1937.

2. (*a*) Millis, G. C., and Conybeare, J. J.: Guy's Hosp. Rep. **74**:369, 1924. (*b*) Susman, W.: J. Path. & Bact. **33**:749, 1930. (*c*) Barnard, W. G.: ibid. **33**:765, 1930. (*d*) Duff, G. L., and Bernstein, C.: Bull. Johns Hopkins Hosp. **52**:67, 1933.

necrosis rather than atrophy, the following 8 cases are added to those already recorded in the literature. The autopsy findings are given in some detail; only brief summaries of the histories are presented.

CASE 1

History.—A 44 year old white woman, unmarried, was well until the spring of 1922, when she suffered a "bilious attack" which lasted for a week. A short time afterward a yellowish brown discoloration of the face and body was noted. This did not involve the scleras, and persisted until death. A second "bilious attack" of one week's duration occurred a few months later. In the interval and until the final attack the patient felt "fairly well" and continued with her work as a dress designer. The final illness, which began one week before death, was ushered in by general weakness and lassitude. This was followed by nausea, severe vomiting and dehydration. The systolic blood pressure was 110 mm. of mercury at the beginning of the last illness but fell considerably during the three days before death. Before leaving for the hospital the patient superintended the packing of her bag but by the time she was carried downstairs she was comatose, and death occurred on the way to the hospital, Feb. 23, 1924. No clinical diagnosis was made.

Autopsy (one hour after death).—The autopsy was restricted to an examination of the abdomen. The skin had a slightly wrinkled appearance, and there was a diffuse brownish pigmentation of the face and to a lesser extent of the body. The subcutaneous fat was reduced in amount. The abdominal organs with the exception of the adrenal glands showed no gross lesions.

Microscopically, the kidneys contained dense focal collections of closely packed lymphocytes just beneath the capsule and in the interstitial tissue of the medulla. The malpighian bodies of the spleen were unusually large and numerous. The gastric mucosa showed many large lymph follicles, some of which had well developed germinal centers. Several small lymph nodes from the adrenal region showed dilated sinuses filled with reticulum cells. No other lesions were found.

Adrenal Glands.—On gross examination no adrenal tissue was found on either side. Blocks were taken from the fatty tissues of both adrenal regions for microscopic study.

Microscopically, there was no trace of adrenal tissue, the only structures encountered being normal ganglions and blood vessels, enclosed by fat and areolar tissue.

CASE 2

History.—A 39 year old white man was admitted to the Toronto General Hospital July 1, 1926 and died July 5, 1926. The patient, a butcher, was well until 1922, when he had "influenza." Subsequent to this he suffered from periodic attacks of weakness, coming about once in three months and lasting usually for three days. One month before admission he noticed severe weakness and "gas on the stomach" and began to vomit after meals. He became progressively weaker so that he was unable to sit up in bed. On admission he appeared ill and dehydrated and showed definite bronzing of the face and the hands. The pulse was feeble and thready, and the blood pressure was 50 systolic and 34 diastolic. The red blood cell count was 7,100,000 and the hemoglobin content 94 per cent. A diagnosis of Addison's disease with addisonian crisis was made. He failed to improve and died quietly in his sleep.

Autopsy (twelve hours after death).—The body was well developed and moderately well nourished and showed patchy brownish pigmentation of the face, the hands and portions of the body. The heart, which weighed 220 Gm., was

extremely flabby, and there was a dark red blotchy subendocardial hemorrhage over the anterior wall of the left ventricle. The gastric mucosa contained many petechial hemorrhages. The spleen weighed 150 Gm., and the malpighian bodies were unusually large. The thymus was not identified, and the lymph nodes showed no enlargement or other abnormality. The thyroid gland, the pituitary gland, the brain and the testes were not examined. The rest of the organs with the exception of the adrenal glands were essentially normal.

Microscopically, the tissues failed to show any additional lesions.

Adrenal Glands.—Because of their very small size, the adrenal glands were located only after a rather prolonged search. The left gland was represented by a small mass of tissue of the approximate shape of an adrenal gland but so thin as to be almost translucent. It was quite firm in consistency, and the cut surface was of a uniform grayish color and failed to show the normal markings. The right gland was even smaller than the left but closely resembled the latter in other respects.

Microscopically, the two glands presented a strikingly similar picture. Over large areas no cortical tissue was present, only a thin strand of medullary tissue and collapsed cortical stroma intervening between the two layers of the capsule. The remaining cortical tissue took the form of irregular masses of cortical cells, infiltrated with lymphocytes and occasional large mononuclear cells and separated by bands of collapsed stroma and newly formed connective tissue. These masses of cortical cells varied in size from 200 to 700 microns and showed no semblance of normal cortical structure and nothing to suggest a zonal arrangement. The individual cells without exception were degenerate in appearance and irregular in size and shape. The majority were considerably larger than normal with indistinct outlines, granular and sometimes vacuolated cytoplasm, and nuclei which were either pyknotic, misshapen, fragmented or absent. Many of the cortical cells were frankly necrotic, and a moderate amount of cellular debris was present (fig. 1 *A*). The vascular channels of the cortex were prominent and engorged with red blood cells. In some areas there were small amounts of blood pigment lying within phagocytic mononuclear cells, but this was not a striking feature. The medulla was small in amount, densely infiltrated with lymphocytes and showed a moderate increase in fibrous tissue. The small pericapsular arteries were thick walled, and although the capsule itself was normal, in several situations collapsed cortical stroma lying immediately beneath it gave an erroneous impression of capsular thickening.

CASE 3

History.—A 31 year old white woman was admitted to the Toronto General Hospital Sept. 24, 1926 and died Oct. 2, 1926. Her previous illnesses included influenza, in 1918, and several abortions. She had had no full term pregnancies. Two years before death she began to complain of weakness and anorexia, which necessitated her staying in bed about one week in four. The "weak spells" persisted and on occasion were associated with nausea and vomiting. In the intervals she was "tired all the time." Nine months before death she first noticed bronzing of the skin, which increased steadily in depth for about a month, until she was quite brown. The final attack of weakness and vomiting began five weeks before death. On admission to the hospital she was poorly nourished, restless and dehydrated. There was brownish pigmentation of the skin, the lips and the buccal mucosa. The pulse was weak and poorly maintained, and the blood pressure was 78 systolic and 58 diastolic. The red blood cell count was 5,200,000 and the hemoglobin content 65 per cent. The Wassermann test of the blood was very strongly positive. No history of treatment for syphilis was obtained.

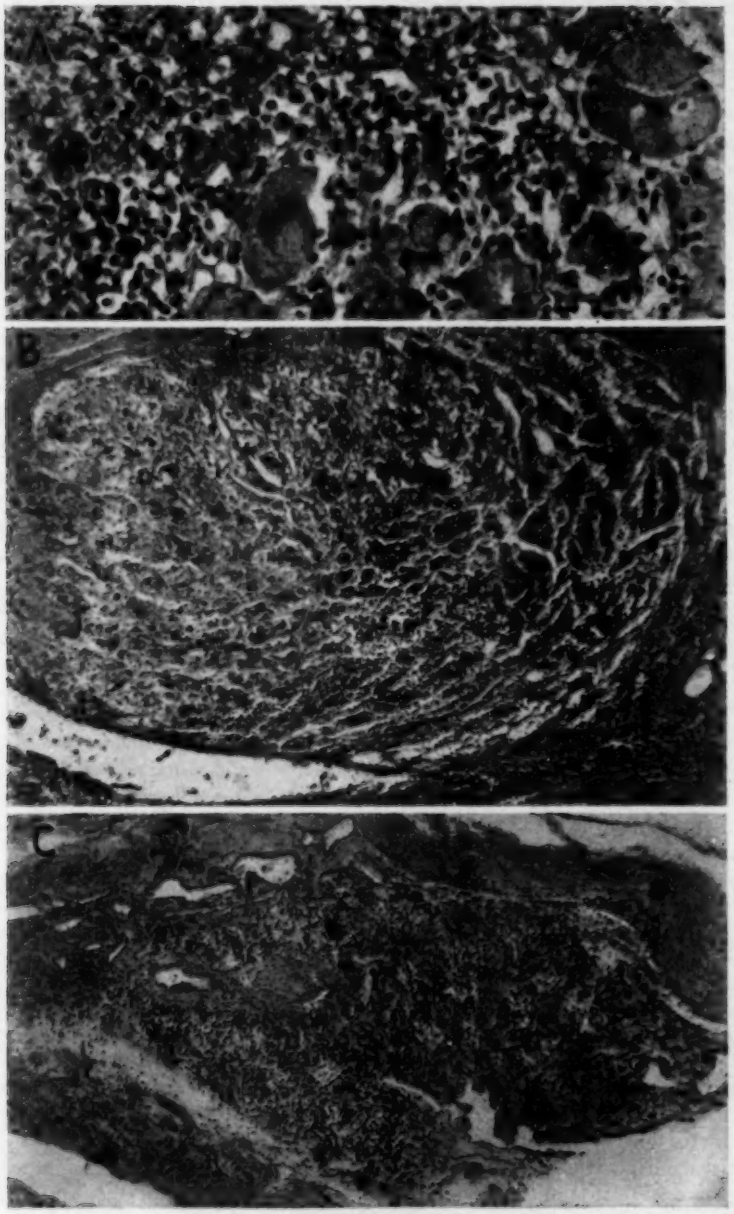

Fig. 1.—*A*, portion of one of the irregular masses of degenerate and necrotic cortical cells in case 2. Hematoxylin and eosin; × 250. *B*, largest of the cortical nodules in case 3. It measured 2.5 mm. in diameter. Hematoxylin and eosin; × 40. *C*, section from remaining adrenal tissue in case 4. A normal amount of medullary tissue lies between the thickened capsule (above) and the central vein (lower left). No cortical tissue is present. Hematoxylin and eosin; × 40.

A diagnosis of Addison's disease and syphilis was made. The vomiting continued, and the patient failed rapidly; the blood pressure dropped to 58 systolic and 38 diastolic three days after admission and was unobtainable for two days before death occurred.

Autopsy (eight hours after death).—The body was greatly emaciated, and all the bony landmarks stood out prominently. There was uniform dark pigmentation of the skin, together with patchy pigmentation of the gums and the soft palate. The thymus was readily identified, although its weight was not recorded. The heart weighed 205 Gm. and the spleen 105 Gm. These were normal in appearance, as were the pituitary gland, the thyroid gland and the ovaries. The fallopian tubes were bound down to the posterior pelvic wall by old adhesions. The subcutaneous and abdominal fat tissue was of a peculiar deep yellow color, verging on orange. The other organs with the exception of the adrenal glands showed no lesions.

Microscopically, the pituitary gland showed much diffuse fibrosis of the anterior lobe with a paucity of cells and a great scarcity of basophils. The normal ratio between acidophils and chromophobes did not appear to be altered. The thyroid gland showed moderate fibrous tissue replacement of glandular elements and marked lymphoid hyperplasia, frequent lymph follicles with well developed germinal centers being present. The acini, which were of normal size, were lined by low cuboidal epithelium and filled with homogeneous colloid. The thymus gland resembled that of an infant in that it was made up of closely packed lymphocytes without fatty replacement. The leptomeninges and the mucosa of the bladder showed focal collections as well as a diffuse infiltration of lymphocytes, while the kidneys also contained small interstitial collections of similar cells.

Adrenal Glands.—Despite a careful search of the retroperitoneal tissues the adrenal glands were not found; however, in microscopic sections of tissue removed from the adrenal regions the glands were identified.

Microscopically, these glands bore a close resemblance one to the other. The larger portion of each gland was composed of compact fibrous tissue infiltrated with lymphocytes, which were distributed both diffusely and in focal collections. Except for two small nodules on each side, no cortical tissue remained. The largest of these nodules (fig. 1*B*) measured 2.5 mm. in the greatest diameter; the other three were considerably smaller but closely resembled the former in other respects. The normal cortical structure was entirely absent, each nodule being made up of jumbled masses of irregularly arranged cells, the whole apparently representing an island of cortical tissue which had escaped complete destruction. Most of the individual cells were well preserved, but many showed irregular cell outlines, coarsely granular cytoplasm and nuclear pyknosis or karyorrhexis. A few showed definite necrosis. These cortical remnants were irregularly infiltrated with lymphocytes but not to the same degree as was the surrounding fibrous tissue. The medulla on each side was moderately abundant and was grouped largely about the central vein. Here, too, there was fairly dense lymphocytic infiltration with increase in mature connective tissue. The medullary cells, although somewhat reduced in number, were of the usual irregularly polygonal shape and of normal appearance. The capsules, which were uniformly thickened, blended almost imperceptibly with the abundant fibrous tissue which made up the major part of the damaged glands.

CASE 4

History.—A 35 year old white woman, married, was first admitted to the Toronto General Hospital June 18, 1933. She complained of weakness, anorexia and darkening of the skin of one year's duration. The patient was poorly

nourished and showed brownish pigmentation of exposed surfaces and pressure points. The blood pressure was 90 systolic and 60 diastolic. A diagnosis of Addison's disease was made, and the patient was discharged feeling somewhat improved and taking 10 Gm. of sodium chloride daily. In July 1934 she was readmitted, unconscious, in hypoglycemic coma. Consciousness was regained after the intravenous administration of dextrose solution. Subsequent hypoglycemic attacks were controlled at home by the taking of dextrose drinks. During the succeeding fifteen months the patient felt generally better than she had since the onset of the illness. The final admission was on Nov. 25, 1935. For the preceding several weeks she had noticed a slow return of severe weakness and ready exhaustion. Two days before admission severe diarrhea and increasing weakness had developed. Examination in the hospital showed an exhausted but conscious deeply pigmented woman. The blood pressure was 90 systolic and 60 diastolic. The blood sugar amounted to 31 mg. and the blood sodium to 280 mg. per hundred cubic centimeters. Dextrose and saline solutions administered intravenously failed to improve her condition, and death occurred Nov. 27, 1935, following a period of coma.

Autopsy (one hour after death).—The body showed moderate emaciation, and there was diffuse light brown pigmentation of the skin, with many dark brown freckles. The pubic and axillary hair was scant. The organs tended to be small, the heart weighing 160 Gm. The retroperitoneal lymph nodes were enlarged, but the other lymph nodes were normal. The kidneys were contracted and had a granular surface, and the ovaries were small and fibrosed. No trace of the thymus gland was present. The thyroid gland was atrophic, weighing only 10 Gm. The pituitary gland was grossly normal, as were the other organs, including the pancreas. The spleen weighed 120 Gm.

Microscopically, the kidneys showed chronic glomerulonephritis with extensive fibrous tissue replacement of parenchymal elements. The thyroid gland showed multiple large foci of lymphocytic hyperplasia and moderate diffuse fibrosis. The anterior lobe of the pituitary gland contained an excessive amount of connective tissue, and there was an almost complete absence of basophil cells, with a reduced number of eosinophils.

Adrenal Glands.—No evidence of adrenal tissue was present in the gross specimen.

Microscopically, adrenal tissue was readily recognized in blocks taken from the adrenal region of each side. On the right side only the capsule with the central vein and a very small amount of adjacent adrenal medulla were present. There was complete absence of cortical tissue. The medulla consisted of only a few normal-appearing chromaffin cells clumped around the central vein. The capsule with its irregular foldings outlined the characteristic contour of a diminutive, very thin adrenal gland. This capsule, however, was uniformly thickened, and except in the area of the central vein there intervened between its two layers only a small amount of fatty areolar tissue, containing an occasional lymphocyte and a few thin-walled vascular spaces. The left gland differed from the right only in its greater content of adrenal medullary tissue. No cortical tissue was present. There was a considerable amount of medulla, which encircled and spread out for some distance on each side of the central vein. The actual volume of medullary tissue did not seem to be greatly reduced, although the contained parenchymal cells were considerably diminished in number. There was a diffuse increase in fine connective tissue in the medullary portion of the gland, together with a greater than normal prominence of thin-walled vascular spaces and a diffuse lymphocytic infiltration (fig. 1 *C*). At each extremity of the left gland large

areas were seen where, as in the right gland and as in cases 3, 5 and 7 (fig. 2 *C*), only a thin layer of areolar tissue separated the two layers of the uniformly thickened capsule.

CASE 5

History.—A 35 year old white woman, married, was first admitted to the Toronto General Hospital Dec. 28, 1934. She gave a history of weakness, loss of weight and darkening of the skin and pigmentation of the lips of seven months' duration. The immediate cause of her admission was acute nasopharyngitis. Her past illnesses included septic abortion, in 1928, and "rheumatism," in 1930. Examination showed in addition to the nasopharyngitis typical addisonian pigmentation and a blood pressure of 85 systolic and 60 diastolic. A diagnosis of Addison's disease was made. While she was in the hospital, the Wassermann reaction of the blood was found to be strongly positive. The blood sodium varied in amount from 291 mg. to 322 mg. per hundred cubic centimeters. The lowest value for blood sugar during fasting was 77 mg. The patient was discharged improved on a high intake of sodium chloride. She remained fairly well until January 1936, when she was readmitted in crisis with an infection of the upper respiratory tract. The blood pressure was 74 systolic and 50 diastolic, and the blood sodium amounted to 290 mg. per hundred cubic centimeters. Despite intensive antisyphilitic treatment in the interval, the Wassermann reaction was still strongly positive. She improved rapidly on intravenous injections of saline solution and extract of adrenal cortex. During a three months' stay in the hospital she received frequent injections of the extract and large amounts of salt by mouth. She seemed to be in satisfactory condition on discharge from the hospital but was readmitted acutely ill four days later, April 18. She died within forty-eight hours with signs of bronchopneumonia.

Autopsy (two hours after death).—The body, which was well nourished, showed diffuse light brown pigmentation of the skin and a scarcity of pubic and axillary hair. There were bronchopneumonia involving the right lung and old inflammatory disease of the fallopian tubes. The thymus gland was not identified, and the lymph nodes showed no enlargement. The heart and the spleen weighed 235 and 190 Gm., respectively. The pituitary gland and the ovaries were normal in size and appearance; the thyroid gland weighed only 20 Gm. but was normal in configuration and consistency. No other gross lesions were present except in the adrenal glands.

Microscopically, the pituitary gland showed the usual proportion of eosinophils, basophils and chromophobe cells and no fibrosis. The thyroid gland exhibited a marked degree of lymphoid hyperplasia and moderate diffuse fibrosis. None of the other organs showed lymphocytic infiltration.

Adrenal Glands.—The left gland weighed 1.1 Gm. and measured 4 by 1.3 by 0.4 cm. The right gland weighed 0.6 Gm. and measured 4 by 2 by 0.2 cm. Except for their difference in thickness, the glands were similar in appearance and of the usual shape. They were yellowish brown, and the cut surface showed a grayish yellow outer layer, which appeared to be the cortex, and a grayish white central portion, which apparently represented the medulla. The cut surface in some areas showed only a grayish white capsule, without either cortex or medulla between its two layers.

Microscopically, the right gland contained a small quantity of cortical tissue and an apparently normal amount of medulla. The latter showed numerous aggregations of lymphocytes and a slight diminution in the number of parenchymal cells with, as in most of the other cases, an increased prominence of thin-walled vascular channels. Otherwise the medulla was normal, the individual polygonal

cells being well preserved and arranged in the usual manner. The cortex was represented by a thin layer, which partially invested the medulla and central vein, and by a small discrete subcapsular nodule, which was completely isolated from the rest of the adrenal tissue by a broad fibrous band (fig. 2*A*). In both situations the cortical tissue was either extremely degenerate or necrotic. None of the usual cortical structure remained, nor was there any trace of a zonal arrangement.

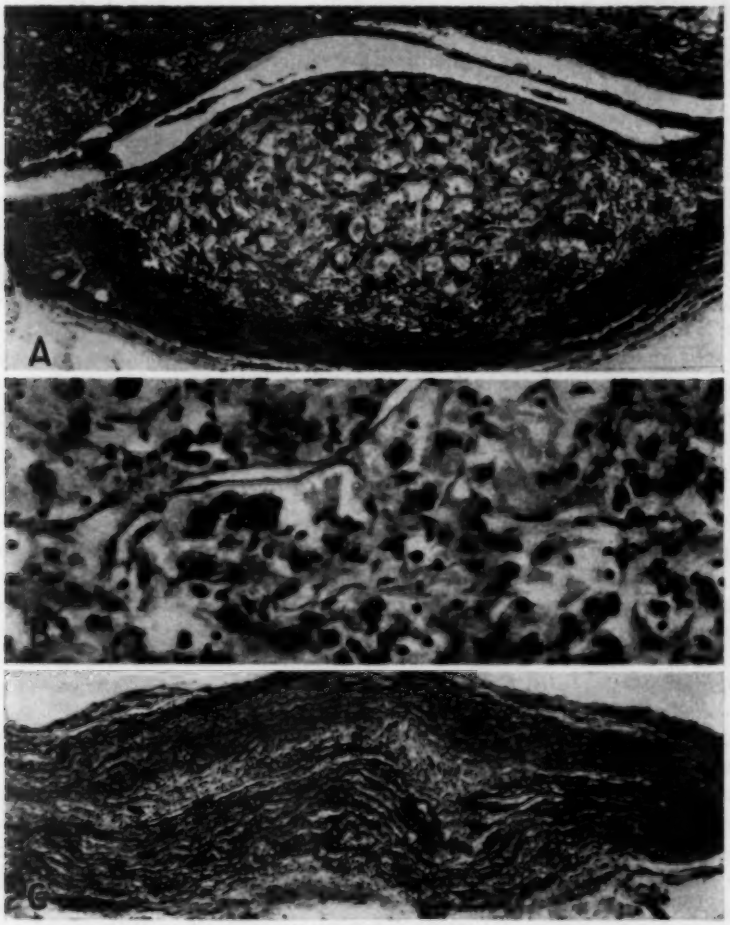

Fig. 2.—*A*, subcapsular cortical nodule in case 5. Hematoxylin and eosin; × 40. *B*, high power view of the degenerate cortical tissue in case 5. Hematoxylin and eosin; × 250. *C*, two layers of the thickened capsule in case 5 separated by only a thin strand of loose connective tissue. Hematoxylin and eosin; × 40.

The cells almost without exception were much larger than normal and ballooned in appearance. In some cases the cell outlines had disappeared and the fused cytoplasm of adjacent cells gave the impression of giant cell formation. The cytoplasm was coarsely granular and in many instances vacuolated, while the nuclei for

the most part were pyknotic, fragmented or absent. A few of them were extremely hyperchromatic and much larger than normal (fig. 2 *B*). In several situations groups of fat cells were present lying among cortical cells, some of which they had apparently replaced. Toward the periphery of the isolated cortical nodule a diffuse infiltration of lymphocytes was seen, while the rest of the cortical tissue contained varying numbers of these cells, both irregularly scattered and in focal collections. In approximately one third of the gland neither cortical nor medullary tissue remained; here the two layers of the capsule were separated by only a thin strand of loose connective tissue (fig. 2 *C*). The left adrenal gland resembled the right in all essentials: The cortex was degenerate and greatly reduced in amount; the medulla was relatively well preserved, and the whole contained varying numbers of lymphocytes.

CASE 6

History.—A 26 year old white woman, married, was first admitted to the Toronto General Hospital May 28, 1939. She gave a history of weakness and ready fatigue of three years' duration and of daily attacks of vomiting for two weeks. She was exhausted in appearance and markedly dehydrated. The skin of the face and the hands was deeply pigmented. The blood pressure was 60 systolic and 40 diastolic. A diagnosis of Addison's disease was made. She improved rapidly following treatment with saline and dextrose solutions and extract of adrenal cortex. She was readmitted to the hospital in crisis twice in 1938 and twice in 1939. One crisis was associated with idiopathic pleurisy, another with menstruation, and still another followed removal of an infected wisdom tooth. In the intervals she felt fairly well. In November 1940 pellets of desoxycorticosterone acetate were implanted in the subcutaneous tissues of the left infrascapular region. The final admission was on Jan. 19, 1941. The patient was admitted in crisis and died a few hours later.

Autopsy (two hours after death).—The body, which was normally developed and moderately well nourished, showed brownish pigmentation and dark freckling of the face and the hands. The significant findings exclusive of those in the adrenal glands consisted of fibrinosanguineous pericarditis, enlargement of the thymus glands, which weighed 20 Gm., enlargement of the cervical, abdominal para-aortic and mesenteric lymph nodes and of Peyer's patches of the ileum, and a thyroid gland which weighed only 15 Gm. but was otherwise normal in appearance. The other organs, including the ovaries, presented no abnormality. The heart weighed 240 Gm. and the spleen 150 Gm. The cranial contents were not examined.

Microscopically, the thyroid gland showed lymphocytic infiltration and diffuse fibrosis. The individual acini, although reduced in number, were of normal appearance with a normal colloid content. The thymus gland was composed of densely packed lymphocytes and showed almost no fatty replacement. The spleen contained numerous, unusually large malpighian bodies with well developed germinal centers, while the liver showed large numbers of lymphocytes in the portal areas. The enlargement of the lymph nodes was seen to be due to marked hyperplasia of the reticulum cells, which occupied mainly the sinuses of the medulla.

Adrenal Glands.—These glands were not identified in situ, but a prolonged search of the regional tissues, after a period of fixation, was rewarded by the finding of a small roughly triangular body on each side. These bodies were of almost identical appearance, each measuring 11 by 5 by 2 mm. Together they weighed 1.0 Gm. Each was composed of what appeared to be a fibrous capsule

enclosing a thin layer of softer grayish tissue. No accessory adrenal tissue was found.

Microscopically, the two adrenal glands resembled each other so closely that the following description is applicable to both. Each was composed entirely of medullary tissue surrounded by a thickened capsule; no vestige of cortex was present in the many sections examined. The striking abnormality of the medulla was the extreme degree of lymphocytic infiltration. For the most part, the infiltrating cells were so closely packed that at first glance the gland resembled a lymph node, and it was only after closer scrutiny that parenchymal cells were seen. In a few areas the adrenal tissue was entirely free of cellular infiltration and relatively normal in appearance, and here, as in other areas where the lymphocytic collections were less dense, numerous chromaffin cells were visible (fig. 3 *A*). The latter were of the usual polygonal shape and showed no degenerative change. An occasional nonmyelinated nerve and a few large ganglion cells were present, while the normal-appearing central vein with its characteristic thick muscle bundles was readily identified. The fibrous tissue comprising the capsule was uniformly increased in amount, but it was not possible to be sure whether this represented a proliferation of capsular connective tissue or merely collapse of the cortical stroma. Apart from slight thickening, the capsular and pericapsular blood vessels showed no abnormality.

CASE 7

History.—A 33 year old white woman, unmarried, was admitted to the Women's College Hospital, Toronto, May 15, 1939. She complained of weakness, fainting attacks and vomiting of two months' duration. There was brown pigmentation of exposed areas and of the buccal mucosa. The blood pressure was 84 systolic and 70 diastolic. A diagnosis of Addison's disease was made, and three months later the patient was discharged improved, to continue with the parenteral administration of extract of adrenal cortex and oral salt therapy. The extract was later replaced by desoxycorticosterone acetate. On this regimen she remained fairly well but never became strong enough to work again as a stenographer. She was readmitted to the hospital Feb. 2, 1940 with pneumonia, from which she made a slow recovery. In May one 150 mg. pellet of desoxycorticosterone acetate was implanted in the subcutaneous tissues of the left infrascapular region. At this time the blood pressure was in the neighborhood of 92 systolic and 68 diastolic. The final admission was on Feb. 26, 1941. The patient complained of severe weakness for three weeks and of vomiting and fainting during the morning of the day of admission. Nasopharyngitis was present, and later hyperpyrexia developed. Despite intravenous therapy and large doses of aqueous extract of adrenal cortex, death occurred five days later.

Autopsy (two hours after death at the Women's College Hospital by Dr. V. Laughlen).—The body was normally developed and fairly well nourished and showed diffuse brownish pigmentation of the skin. The thymus gland was not identified, and the lymph nodes were not enlarged. The ovaries were normal in size and appearance, as was the heart, which weighed 225 Gm. The spleen weighed 170 Gm. and was likewise normal. A small accessory spleen was present. The thyroid gland and the cranial contents were not examined. The other organs apart from the adrenal glands showed no lesions.

Microscopically, there were no additional observations. With the exception of the right adrenal gland, none of the organs showed lymphocytic infiltration.

Adrenal Glands.—These glands were identified with difficulty because of their small size. The left was slightly larger than the right, but the two together made

up not more than a small fraction of a normal gland. No golden brown cortical tissue was visible in either, the cut surfaces of each being of a homogeneous dull grayish color.

Microscopically, no cortical tissue remained on the left side, the gland being made up of medullary tissue enclosed by a normally thin capsule. The medulla, which did not seem to be reduced in amount, contained a normal central vein, a

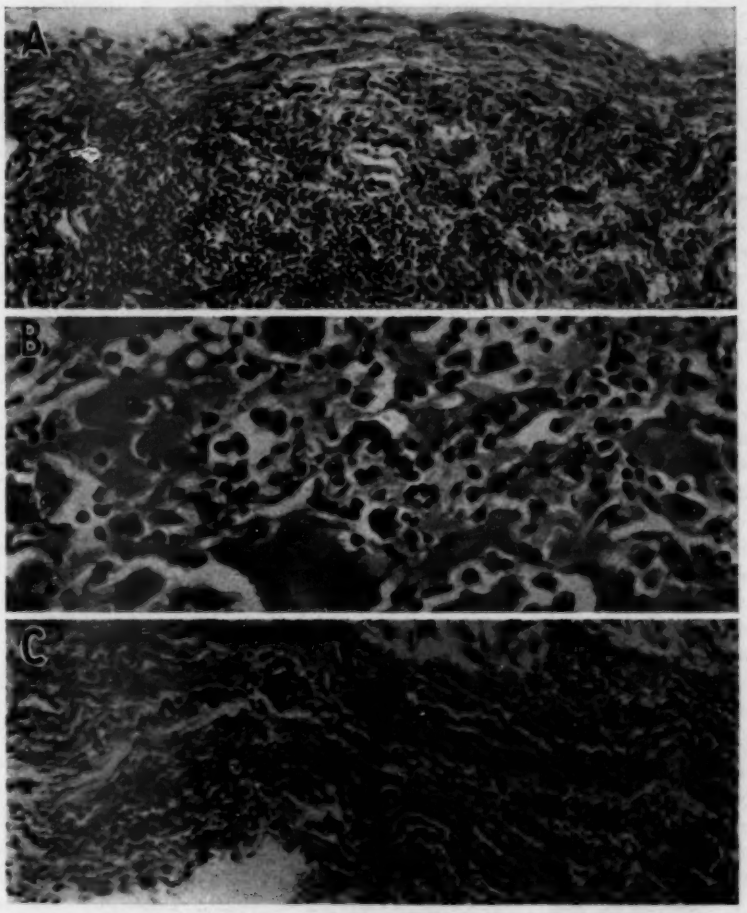

Fig. 3.—*A*, absence of cortical tissue and extreme degree of lymphocytic infiltration of the medulla in case 6. Hematoxylin and eosin; × 110. *B*, degeneration and necrosis of cortical cells in case 7. Hematoxylin and eosin; × 250. *C*, capsule of the left adrenal giand (case 8)—all that remains of this gland. Hematoxylin and eosin; × 200.

few nonmedullated nerves and ganglion cells. The individual cells of the medulla, although not altered in appearance, seemed to be somewhat reduced in number. Thin-walled vascular channels were more prominent than in a normal gland. There was neither fibrosis nor lymphocytic infiltration. The right gland showed small

numbers of cortical cells at one end but no other cortical tissue. No vestige of normal cortical structure remained. The few cortical cells were separated by collapsed stroma and without exception showed severe degenerative changes or actual necrosis. The nuclei, for the most part, were pyknotic and misshapen, and the cell boundaries were indistinct. There was diffuse lymphocytic infiltration of this residual cortical tissue (fig. 3 *B*). In the medulla scarcity of parenchymal cells, increase in fibrous tissue and rather dense lymphocytic infiltration were apparent. The capsule was moderately thickened, and in some situations only a few strands of loose connective tissue and focal collections of lymphocytes intervened between its two layers.

CASE 8

History.—A 16 year old white youth was first admitted to the Toronto General Hospital in December 1937. His complaints were weakness, loss of appetite and intermittent attacks of nausea and vomiting since starting to work some five months previously. Examination revealed a slightly built, emaciated and mentally dull adolescent male. The other significant findings included persistent hypotension (the blood pressure varying from 52 systolic and 25 diastolic to 100 systolic and 70 diastolic), a slight degree of diffuse brownish pigmentation of the skin of the forehead and the elbows, and a blood sodium content of 270 mg. per hundred cubic centimeters. The tuberculin test was negative. Roentgen plates of the adrenal regions showed no abnormality. A diagnosis of Addison's disease due to simple atrophy of the adrenals was made. With large doses of sodium chloride by mouth and aqueous extract of adrenal cortex intramuscularly, the patient showed marked improvement and gain in weight. He was discharged in April 1938 and continued to take the salt and the extract. In September he was readmitted in a severe addisonian crisis precipitated by a cold in the head and sore throat. The condition was controlled by intravenous injections of hypertonic saline solution and with extract of adrenal cortex, which was later replaced by desoxycorticosterone acetate. The patient carried on, feeling moderately well but not strong enough to work, until January 1940, when he was admitted to the hospital in a semicomatose state with nasopharyngitis. The blood sugar amounted to 27 mg. per hundred cubic centimeters, and a diagnosis of hypoglycemic reaction was made. This was controlled satisfactorily by intravenous administration of dextrose. A similar attack occurred in July 1941, when, following the development of a sore throat, the patient missed two or three meals. Shortly thereafter he was admitted to the hospital unconscious, with a blood sugar determination of 33 mg. per hundred cubic centimeters. Consciousness was promptly regained following intravenous administration of dextrose, and the patient was discharged a few days later. Three days before the final admission he experienced general malaise and loss of appetite, and early in the morning of the day of admission (November 18) he was found in an unconscious state. On admission he was moribund, and signs of bronchopneumonia were present. Death occurred a few hours later.

Autopsy (three hours after death).—The cadaver was that of a well developed but emaciated youth. The pigmentation, which was not marked, corresponded to that which had been observed clinically. The axillary and the pubic hair were sparse, but the genitalia showed normal development. The lungs were heavier than normal, and clear fluid could readily be expressed from their cut surfaces. The lower lobe of the right lung contained scattered small areas of pneumonic consolidation. The thyroid gland, which weighed 25 Gm., was somewhat tougher and more fibrous than normally. The thymus gland was a bilobed mass of firm pinkish white tissue, weighing 15 Gm. The abdominal lymph nodes were definitely

enlarged, pale, soft and succulent. They measured up to 2 cm. in greatest diameter. The pituitary gland was normal on gross examination and weighed 0.5 Gm. The brain was edematous and increased in weight. Apart from the adrenal glands, the other organs, including the heart and the spleen, which weighed 260 and 225 Gm., respectively, were normal.

Microscopically, the lungs showed marked confluent bronchopneumonia involving both lower lobes and edema and patchy small areas of pneumonic change in the upper lobes. The thyroid gland exhibited marked fibrosis; broad bands of scar tissue separated individual lobules and parts of lobules, the component acini of which were formed by flattened cells and contained much colloid. Extremely large and numerous areas of lymphoid tissue replacement of thyroid tissue proper were seen. In these situations there were closely packed lymphocytic collections, measuring up to 1 mm. in diameter. Some of these showed "germinal centers." The thymus gland showed only slight fatty replacement and, the patient's age being taken into consideration, was definitely hyperplastic. The only notable finding in the liver was moderate infiltration of the portal areas with lymphocytes. The abdominal and the thoracic lymph nodes presented a picture of "sinus catarrh," the cortical and the medullary sinuses being filled with proliferating reticulum cells. In the perineural sheaths of some of the larger retroperitoneal nerves there were closely packed lymphocytes, although there was no suggestion of any inflammatory or degenerative lesion involving these structures. The testes showed normal spermatogenesis and some degree of hyperplasia of the interstitial cells, moderately large islets of these cells being scattered at intervals between the tubules. The interstitial connective tissue was somewhat increased in amount. The pituitary gland presented diffuse fibrosis of the anterior lobe, with considerable diminution in the number of parenchymal cells, so that the volume of secretory tissue was significantly reduced. The normal ratio between chromophobe and acidophil cells was not altered, but basophil cells were infrequently encountered and were practically confined to the extreme posterior part of this lobe. The posterior lobe was normal. Sections of the brain confirmed the presence of edema. A section of abdominal skin showed an increased number of melanophores in the cutis vera and a slight increase of the melanin content of the basal layer of the epidermis. The other organs, including the heart, the kidneys, the spleen and the pancreas, were histologically normal.

Adrenal Glands.—Despite an intensive search with the retroperitoneal organs in situ, no trace of adrenal tissue was found. The aorta was laid open, and the adrenal arteries on the two sides were identified with difficulty because of their diminutive size. It was possible to trace them for only a short distance into the periadventitial areolar tissue of the aorta, where they disappeared and could be followed no farther. No adrenal branch could be demonstrated arising from either the inferior phrenic or the renal artery on either side. A block of tissue extending from the lower part of the thoracic aorta to the origin of the inferior mesenteric artery and including the diaphragm, the kidneys, the adrenal fatty tissues, the inferior vena cava and all the fatty areolar tissue of the fossae renales and upper lateral abdominal gutters was then taken. This was painstakingly dissected on repeated occasions for a period totaling six hours without any grossly recognizable adrenal tissue being encountered. A small flattened scarred area, the component tissues of which had a volume of approximately 0.5 cc., was encountered in the adrenal region on each side, and both of these were preserved for serial microscopic sectioning in the belief that they probably represented remnants of the adrenal glands.

Microscopically, the thickened area from the right side was found to be only patternless scar tissue. No trace of adrenal tissue was identified in it or in any of the blocks taken from that side. The area of thickening from the left side proved to be all that remained of the left adrenal. Only the wavy, somewhat thickened capsule persisted, but it outlined unmistakably the characteristic contour of an adrenal gland (fig. 3 *C*). Serial microscopic sections showed no parenchymal tissue of either cortical or medullary type; only fatty areolar tissue containing small blood vessels and occasional scattered lymphocytes was present within the fibrous envelope of the gland.

COMMENT

Clinically, these cases are noteworthy for their remarkable similarity in regard to symptoms and disease progress. Six of the 8 patients were women; the youngest was 20 and the oldest 44 at the time of death. All the patients suffered from gradually developing and slowly progressive weakness and asthenia interspersed with attacks of prostration and vomiting. All had some degree of hypotension, and pigmentation developed in all. All died in an Addisonian crisis between two and four years after the onset of symptoms.

Adrenal Lesions.—The lesions encountered in the adrenal glands presented the same striking similarity from case to case as did the clinical course of the patients. No parallelism could be found, however, between the duration of symptoms and the severity of the lesions.

In cases 1 and 8 the process had reached an end stage; no glandular tissue was found. It is possible that in case 1 a more painstaking search would have revealed remnants of the adrenal gland, especially so in view of the difficulty with which adrenal tissue was found in several of the other cases. The experience in case 8, however, in which a most exhaustive search demonstrated that all glandular tissue had disappeared, as well as the failure of several other observers [3] to find persisting glandular tissue, inclines me to the belief that in case 1, as in case 8, no glandular tissue existed at the time of death.

In the other 6 cases the significant abnormalities involved the cortex. In cases 4 and 6 there was complete absence of cortical tissue. In case 7 the amount of residual cortex was limited to a few isolated cells, while in cases 2, 3 and 5, although cortical tissue was readily identified, the destructive process was extremely far advanced, the viable tissue having been reduced to a small fraction of the normal. In these 4 cases in which cortical tissue remained, the picture was one of degeneration and necrosis with resultant reduction in cortical volume. In the other 2 cases the process of cortical destruction had reached an end stage; only collapsed cortical stroma intervened between the wrinkled capsule and the medullary tissue. Nothing was found to suggest that the lesions

3. Brenner, O.: Quart. J. Med. **22**:121, 1928 (table summarizing cases at conclusion of article).

might have been of ischemic or tuberculous origin. A consistent feature of all cases except 1 and 8, which have been considered separately, was the relatively close resemblance of the one adrenal to its fellow of the opposite side, each containing approximately the same amount of residual cortex. An additional constant finding, which has been frequently noted by previous writers,[4] was the marked degree of lymphocytic infiltration of the medulla and of the degenerate cortical tissue. In contrast to the cortex, and despite the lymphocytic infiltration, the medulla was relatively well preserved and contained probably not far from its normal complement of chromaffin cells. This marked loss of cortical tissue with preservation of the medulla in 6 of the 8 cases is in agreement with the accumulating clinical and experimental evidence which indicates that Addison's disease from any cause is the result of cortical damage and that damage to the medulla plays at most a minor role in its production.

A study of the process which results in the great reduction in volume of cortical tissue indicates that it is clearly not one of simple atrophy in the restricted sense of the term, for although the gland is unquestionably smaller than normal, it does not shrink because of atrophy of its individual cells but because of destruction and disappearance of these cells. Neither is the process hypoplasia as has been suggested by some writers; the degenerative changes in the cortical cells, together with the wrinkling of the capsule and the disruption of the cortical structure, point to an acquired lesion. Further, as Guttman [4a] has indicated, it is extremely difficult to conceive of a congenital underdevelopment of which no symptoms are present until as late as the fourth or the fifth decade. Although the almost invariable finding of lymphocytic infiltration of the adrenal glands in the present and other series of cases might suggest that the process is inflammatory, no other feature suggestive of inflammation is found. Areas of lymphocytic infiltration in otherwise normal adrenal glands are encountered by no means infrequently in routine autopsies. Moreover, collections of lymphocytes such as those present in the adrenal glands in the cases under consideration, when seen, as they so commonly are, in such conditions as nephrosclerosis and hepatic cirrhosis, are not interpreted as being inflammatory in origin but rather as accompanying the process of degeneration. Although this progressive and bilateral destructive process involving especially the adrenal cortex suggests the action of some damaging substance or substances transported by the blood stream, the nature of the hypothetic agents is completely unknown. An analogy has been drawn by Wells [4b] between the destructive process involving the adrenal cortex in cases of "adrenal atrophy" and diffuse toxic necrosis of the liver. Indeed, apart from an

4. (*a*) Guttman, P. H.: Arch. Path. **10**:742 and 895, 1930. (*b*) Wells, H. G.: ibid. **10**:499, 1930. Susman.[2b]

apparent dissimilarity in tempo, the two processes seem to differ in no essential respect. In the same sense as the term "subacute necrosis of the liver" is preferable to "subacute yellow atrophy," so is the term "necrosis of the adrenal cortex" preferable to the term "adrenal atrophy."

Associated Findings.—Several reports [5] have indicated that a small heart is to be expected in cases of Addison's disease, especially in those in which the disease is due to "atrophy." In the 7 cases in which the heart was examined, the average weight was 220 Gm., a weight not less than that to be expected in any case of prolonged wasting disease. The other organs, generally speaking, were smaller than usual, in keeping with the patients' state of poor nutrition.

The majority of articles dealing with Addison's disease from the standpoint of pathology have recorded varying degrees of hyperplasia of lymphoid tissue throughout the body. In all cases in the present series with the exception of case 7 there was some degree either of lymphoid hyperplasia or of lymphoid infiltration of organs. There was generalized hyperplasia of lymph nodes in case 6, together with non-involution of the thymus gland and enlargement of Peyer's patches. In cases 3 and 8 also the thymus gland was large and hyperplastic, while in cases 4 and 8 many of the lymph nodes were considerably increased in size. Enlargement of the malpighian bodies of the spleen was seen in 3 cases. Lymphocytic infiltration of organs, described by Duff and Bernstein,[2d] was observed in the present series. In 2 cases the organs involved were the kidneys, and 1 of these cases showed in addition round cell infiltration of the meninges. In another case there was lymphocytic infiltration of the portal areas of the liver and of the sheaths of several of the retroperitoneal nerves. In all 5 cases in which the thyroid gland was examined, it exhibited large, well developed lymph nodules with prominent, active-appearing germinal centers. The significance of these findings is difficult to evaluate, the more so because of the relatively frequent occurrence of one or more of them in the same case in routine autopsy material.

Of the 5 thyroid glands examined, only 1 was of average size. The other 4 were small, 2 weighing 20 Gm. each and the 2 others 15 and 10 Gm., respectively. Microscopically, the striking lesion in all 5 was the remarkable degree of lymphoid infiltration and lymphoid hyperplasia. In 1 case these features were so marked as to suggest at first sight a lymphadenoid goiter. An additional constant feature was fibrosis and fibrous tissue replacement of parenchymal elements. In no instance was there any sign of epithelial hyperplasia, the acini being for the most part small, lined by low cuboidal or flattened epithelium and filled with

5. Susman,[2b] Brenner.[3]

homogeneous deep-staining colloid. These characteristics of the thyroid gland in Addison's disease—atrophy, lymphoid infiltration and fibrosis—have been previously remarked on by Brenner,[2a] Susman [2b] and others.

The pituitary gland was grossly normal in all 4 cases in which it was examined, and in 1 of these cases no microscopic abnormality was found. In the other 3 cases (3, 4 and 8), however, similar lesions were presented: a diffuse increase of fine connective tissue in the anterior lobe and a great scarcity of basophil cells, the latter being represented by an occasional cell at the extreme posterior end of the pars glandularis. In cases 3 and 4 these few remaining basophil cells showed nuclear pyknosis and vacuolation of the cytoplasm, indicating a degenerative process. The chromophobe and eosinophilic cells appeared to be reduced in numbers in these cases as well as in case 8. In none of the 4 cases was a differential cell count made, although in each case granule stains were employed. The increased connective tissue content and the diminution in the number of basophil cells are in agreement with the findings of Crooke and Russell [6] in their study of the pituitary glands in a series of cases of Addison's disease. The abnormal transitional forms described by these workers, however, were not recognized in the present material.

In 1 case the fat tissue of the body was described as being of a peculiar dark yellowish brown color, and this same unusual pigmentation of fat, a finding which to my knowledge has not been previously recorded in this condition, was seen in 3 of the last 4 cases of Addison's disease due to adrenal tuberculosis coming to autopsy in this department. At the time of autopsy the possibility that the coloration was due to carotene or an allied fat-soluble pigment was considered. Despite the fact that several cases of carotenemia must have come to autopsy in this department not one member of the department recalled having previously seen fat tissue comparable in color with that observed in these cases as would be expected if the pigment in this instance had been carotene. Analysis of portions of subcutaneous and abdominal fat tissue in the 3 cases of Addison's disease due to tuberculosis mentioned revealed only a slightly higher than average carotene and carotenoid content. In view of the evidence that the pigmentation was probably due neither to carotene nor to a fat-soluble substance of similar nature, the possibility that it may have resulted from the deposition of melanin in low concentration must be kept in mind. No investigation of the tissue was carried out with a view to determining its possible content of melanin.

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6. Crooke, A. C., and Russell, D. S.: J. Path. & Bact. **40**:255, 1935.

SUMMARY

Eight cases of Addison's disease due to a nontuberculous destructive process of the adrenal cortex are reported. In 6 cases the lesion was one of cortical destruction, with severe and widespread necrosis of the cortex in 4 cases and complete disappearance of cortical tissue in the other 2. In each of these 6 cases the adrenal medulla was comparatively well preserved but constantly showed lymphocytic infiltration. In 2 additional cases no adrenal parenchymal tissue remained, although the process in these cases was presumed to be of the same nature as that in the other 6.

The thyroid gland was atrophic, fibrosed and infiltrated with lymphocytes. The pituitary gland in 3 of 4 cases in which it was examined showed a diffuse increase in the connective tissue of the anterior lobe and a great scarcity of basophil cells. The eosinophilic and chromophobe cells were also reduced in number.

A hitherto unrecorded pigmentation of fat tissue is reported.

The findings indicate that the process in the adrenal glands is not one of true atrophy, a term under which cases of this nature have popularly been described, but rather one of disappearance of tissue consequent on cortical necrosis. No clue as to the cause of the cortical necrosis was obtained.

The incidence of this pathologic process in the present series of cases of Addison's disease was 41 per cent.

STUDIES OF NORMAL AND OF ABNORMAL MITOTIC ACTIVITY

II. THE RATE AND THE PERIODICITY OF THE MITOTIC ACTIVITY OF EXPERIMENTAL EPIDERMOID CARCINOMA IN MICE

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Autonomy of growth is one of the principal characters of cancer. Growth in both neoplasms and normal tissues is due principally to mitotic cellular division. The rate and the periodicity of the mitotic activity of normal tissue are ultimate expressions of the factors which regulate that activity. A comparison of cancer and the normal tissue from which it is derived with respect to rate and periodicity of mitotic activity may shed light on certain fundamental characters of the cancer cell.

The literature reveals a single study along this line. Dublin, Gregg and Broders [1] removed two specimens of cancerous growth in each of 5 cases of carcinoma of the large intestine of man, one between 10 a. m. and 12 m., and one between 10 and 12 p. m. In each specimen they counted 3,000 to 5,000 tumor cells and established as a mitosis coefficient the number of mitoses per thousand of tumor cells. The mitosis coefficients with the exception of that for one specimen were essentially the same morning and night. They deduced that mitotic rhythm was absent. No control specimens were examined. This deduction is not warranted, for it is based on the assumption that the mitotic activity of colonic epithelium duplicates that of human epidermis, which has been shown to be minimum in the morning and maximum at night.[2] The curve of mitotic activity for one tissue of an organism is not necessarily similar to that for another; in fact, the curves for rat renal cortex, submaxillary salivary gland and epidermis are quite different.[3] In human colonic epithelium maximum and minimum mitotic activity may well occur at times other than 10 a. m. to 12 m. and 10 to 12 p. m.

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1. Dublin, W. B.; Gregg, R. O., and Broders, A. C.: Proc. Staff Meet., Mayo Clin. **15**:623, 1940; Arch. Path. **30**:893, 1940.

2. Cooper, Z. K., and Schiff, A.: Proc. Soc. Exper. Biol. & Med. **39**:323, 1938. Cooper, Z. K.: J. Invest. Dermat. **2**:289, 1939. Broders, A. C., and Dublin, W. B.: Proc. Staff Meet., Mayo Clin. **14**:423, 1939.

3. Blumenfeld, C. M.: Arch. Path. **33**:770, 1942.

If deductions concerning the rate and the rhythm of mitotic activity in tumors are to be valid, certain variables must be controlled as rigidly as possible. They include the type, differentiation, location, size and age of the tumor and the species, strain, sex and age of the host. The use of tumors experimentally produced in animals permits a degree of control of these variables practically impossible of attainment in man.

MATERIALS AND METHODS

Mice of the CBA strain, bred from animals provided by Dr. L. C. Strong, of the department of anatomy of Yale University, were used because the incidence of spontaneous tumors in them is negligible. The carcinogen was methylcholanthrene, applied as a 0.3 per cent solution in benzene. The experiment was begun with 65 male mice 2 months old. The solution was applied with a no. 6 camel's hair brush, two strokes being made twice weekly in the interscapular region. In fourteen to fifteen weeks, with remarkable regularity, grossly discernible tumors, which proved to be cancerous, appeared in most of the mice. Usually there were several cancers as well as benign papillomas in the painted area. As a check on the nature of the apparent cancers, 1 mouse from each of 6 litters was set aside at the end of fourteen or fifteen weeks. The lesions in each of these mice without further application of carcinogen progressed, coalescing or remaining separate, to form single or multiple large ulcerated masses, the seat of extensive necrosis and suppuration. The remaining animals were observed daily. When a tumor judged grossly to be a cancer reached a maximum diameter of 1 cm., the animal bearing it was killed. The average interval from the first application of the carcinogen to autopsy was one hundred twenty-three and seven-tenths days. The entire painted area and a piece of normal skin from the ventral central abdominal region were removed, fixed at once in Bouin's fluid, embedded in paraffin, sectioned at 8 microns and stained with hematoxylin and eosin. Animals were killed at 8 a. m., 12 m., 4 p. m., 8 p. m., 12 p. m. and 4 a. m. Members of a litter were distributed as evenly as possible through the twenty-four hour period to minimize the effect of inherent differences in mitotic activity. In this manner test and control specimens were obtained from 60 mice, ten for each period of time. The tumors used were all diagnosed as well or moderately well differentiated squamous cell carcinoma. They showed partial or complete lack of cell stratification and irregular keratinization, and some of them, loss of intercellular bridges. Pleomorphism of cells was moderate to marked, with occasional giant multinucleated forms. There were numerous abnormal mitoses, including multipolar, unequal and widely dispersed forms. All tumors showed invasion of the dermis and direct extension into the subcutaneous layer; many, direct extension into or through the panniculus carnosus. Neither regional nor remote metastasis was observed, which may have been due to the nature or to the short period of existence of the tumor or to both. Degeneration, necrosis, ulceration and suppuration of the tumors were common, and such areas were avoided in counting mitoses. Occasionally, fibrosarcoma developed in juxtaposition to the carcinoma, presumably because ulceration afforded the carcinogen access to the dermis.

Mitotic activity was determined by counting the mitoses in 500 fields, as explained in a previous article.[8] Each field measured 60 by 70 microns. The sections were cut perpendicular to the cutaneous surface, at a thickness of 8 microns. Sections of tumor readily afforded 500 fields, each 60 by 70 microns. Sections of

normal epidermis did not fill the field. Instead, 500 segments of epidermis, each 60 microns long, 8 microns thick and of varied width, were studied. Therefore, the volume of tumor studied was greater than that of epidermis. Provided the sample of each specimen studied was representative of the whole, this difference in volumes will not affect conclusions concerning the presence or the absence or the character of the diurnal variation or of the periodicity of the mitotic activity of the normal and of the cancerous epidermis; it will, however, make impossible a comparison of the rates of the mitotic activity of normal epidermis and the tumor. For such a comparison a determination of the rate of mitosis per cell would be best, but there are certain difficulties of technic and interpretation which deserve mention. Indistinct cell limits would necessitate counting nuclei rather than cells in normal epidermis and in carcinoma, which would result in a determination of the rate of mitosis per nucleus. Enumeration of each nucleus of multinucleated tumor cells would introduce an error. Keratinized cells of normal epidermis do not exhibit mitosis; yet it is not possible to distinguish clearly epidermal cells capable of undergoing mitotic division, which should, therefore, be counted, from cells in which mitosis cannot occur. Factors such as the production of less or more than two daughter cells by abnormal mitosis, the duration of mitosis, the length of the intermitotic interval and the life period of a cell, all of which should be considered, cannot be determined from the material used in this study. Because of these difficulties it was decided to express rate of mitosis as the number counted in 500 fields. For the tumor, this amounted to an expression of the number of mitoses in 500 portions, each 60 by 70 by 8 microns, or 0.0168 cu. mm. of tumor. For normal epidermis, the volume varied with the width of epidermis in each specimen. The average width was determined for each specimen by measuring every fiftieth field counted, with an ocular micrometer. The volume of the observed portion of each specimen of normal epidermis was calculated, and from this and the number of mitoses counted, an estimate was made of the number of mitoses in a volume equal to the volume of tumor studied. For example, in the specimen of normal epidermis of mouse F2 the average width of the epidermis was 7 microns. The volume of epidermis studied was 500 (60 by 8 by 7 microns) cubic microns or 0.00168 cu. mm. The number of mitoses observed was 10. The volume of the epidermis being one tenth of the volume of the tumor studied, multiplication of the observed number of mitoses by 10 permits a comparison of the numbers of mitoses in equal volumes of epidermis and tumor. The values thus calculated, i. e., the numbers of mitoses in volumes of epidermis equal to the volumes of tumor, are referred to as corrected values. Fisher's [4] small sample methods have been employed in the statistical analysis of the data obtained. In tables 2 and 3, containing statistical data, the symbol t is a significance ratio. With it, P is determined according to degrees of freedom. P is an expression of the likelihood of occurrence of a difference the same as that observed by chance. A P of 0.01 means this chance is 1 of 100; a P of 0.6, 60 of 100. Conventionally, a P of 0.05 or less is interpreted as indicating statistical significance.

RESULTS

Normal Epidermis.—The averaged values listed in table 1 and represented as a curve in the accompanying chart show a diurnal rhythm of mitotic activity. Maximum mitotic activity occurred at 8 a. m. to

4. Fisher, R. A.: Statistical Methods for Research Workers, ed. 7, London, Oliver & Boyd, 1938.

TABLE 1.—*Individual and Average Numbers of Mitoses per Five Hundred Fields*

8 a. m.	12 m.	4 p. m.	8 p. m.	12 p. m.	4 a. m.
		For Epidermis			
22	75	30	18	8	15
7	64	45	22	11	20
32	6	13	1	8	23
13	37	20	18	8	6
17	57	6	1	6	11
14	10	2	8	2	12
10	28	6	9	2	6
10	11	1	3	10	6
28	27	13	1	2	19
24	50	9	10	2	6
17.7	37.4	16.5	9.1	5.9	12.4
		Corrected Values for Epidermis			
97	338	156	67	62	77
59	384	167	121	68	112
365	67	86	7	87	129
142	278	136	131	69	51
156	342	46	13	55	90
129	74	152	55	21	82
100	314	61	76	25	55
109	99	15	32	109	55
274	232	122	8	18	158
266	325	82	80	16	59
109.7	245.3	102.3	59.9	53.0	86.8
		For Carcinoma			
96	260	127	96	123	155
214	136	201	335	341	159
109	115	212	176	149	222
169	222	192	177	164	233
131	371	226	168	268	255
526	262	257	144	190	223
114	294	205	272	221	170
265	204	258	304	177	188
228	193	260	184	220	211
242	332	138	291	225	339
209.1	240.9	207.6	214.7	207.8	215.5

TABLE 2.—*Statistical Data on the Significance of the Difference Between the Average Values of Mitotic Activity at Various Periods of Time*

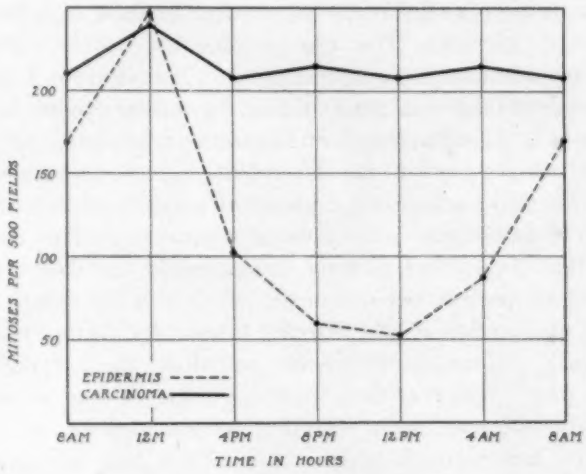
	Corrected Values for Epidermis			Carcinoma		
Times Compared	Diff. ± S. E.	t	P	Diff. ± S. E.	t	P
8 a.m. vs. 12 m.	75.6 ± 49.3	1.532	0.2-0.1	31.8 ± 48.2	0.660	0.6-0.5
8 a.m. vs. 4 p.m.	67.4 ± 35.2	1.917	0.1-0.05	1.5 ± 42.8	0.035	0.9
8 a.m. vs. 8 p.m.	109.8 ± 34.2	3.209	0.01	5.6 ± 47.4	0.118	0.9
8 a.m. vs. 12 p.m.	116.7 ± 32.7	3.569	0.01	1.3 ± 44.9	0.028	0.9
8 a.m. vs. 4 a.m.	82.9 ± 33.1	2.505	0.05-0.02	6.4 ± 43.7	0.146	0.9-0.8
12 m. vs. 4 p.m.	143.0 ± 41.1	3.475	0.01	33.3 ± 30.5	1.093	0.3-0.2
12 m. vs. 8 p.m.	185.4 ± 40.9	4.535	0.01	26.2 ± 36.6	0.716	0.5-0.4
12 m. vs. 12 p.m.	192.3 ± 39.6	4.858	0.01	33.1 ± 33.2	0.996	0.4-0.3
12 m. vs. 4 a.m.	158.5 ± 39.9	3.968	0.01	25.4 ± 31.4	0.809	0.5-0.4
4 p.m. vs. 8 p.m.	42.4 ± 21.7	1.950	0.1-0.05	7.1 ± 29.1	0.243	0.9-0.8
4 p.m. vs. 12 p.m.	49.3 ± 19.3	2.557	0.02-0.01	0.2 ± 24.8	0.008	0.9
4 p.m. vs. 4 a.m.	15.5 ± 19.5	0.796	0.5-0.4	7.9 ± 22.8	0.347	0.8-0.7
8 p.m. vs. 12 p.m.	6.9 ± 17.5	0.393	0.7-0.6	6.9 ± 32.0	0.215	0.9-0.8
8 p.m. vs. 4 a.m.	26.9 ± 18.3	1.470	0.2-0.1	0.8 ± 30.5	0.026	0.9
12 p.m. vs. 4 a.m.	33.8 ± 15.2	2.216	0.05-0.02	7.7 ± 26.0	0.296	0.8-0.7

Diff. = Difference between average values.
S. E. = Standard error of the difference between average values.
t = Significance ratio, obtained by dividing the difference between average values by the standard error of the difference between average values.
P = An expression of the likelihood of occurrence by chance of a difference the same as that observed. It is obtained from t according to the number of cases in the series compared. P of 0.01 means this chance is 1 of 100; P of 0.6, 60 of 100. Conventionally, P of 0.05 or less is interpreted as indicating statistical significance.

12 m.; minimum mitotic activity, at 8 p. m. to 12 p. m. The corrected values for epidermis show more than four times as many mitoses at noon as at midnight. The statistical studies recorded in table 2 show that the difference is significant. The character of the curve is similar to

TABLE 3.—*Comparison of Mitotic Activity in Epidermis (Corrected Values) with Mitotic Activity in Carcinoma*

Time	Epidermis vs. Carcinoma		Diff. ± S. E.	t	P
8 a.m.	169.7	209.1	39.4 ± 50.8	0.775	0.9
12 m.	245.3	240.9	4.4 ± 46.6	0.094	0.9
4 p.m.	102.3	207.6	105.3 ± 22.1	4.764	0.01
8 p.m.	59.9	214.7	154.8 ± 28.9	5.358	0.01
12 p.m.	53.0	207.8	154.8 ± 22.4	6.917	0.01
4 a.m.	86.8	215.5	128.7 ± 20.7	6.215	0.01



Mitotic activity at four hour intervals for a period of twenty-four hours in experimental epidermoid carcinoma and in an equal volume of normal epidermis. Average numbers of mitoses were plotted.

that obtained for rat epidermis,[3] and the demonstration of periodicity is in agreement with the findings of Ortiz Picón [5] and Cooper and Franklin.[6] Carleton [7] also demonstrated periodic mitotic activity in mouse epidermis, but the maximum and minimum periods were at opposite times. No explanation is available for this difference.

Carcinoma.—Individual values and the averages are listed in table 1, statistical data in tables 2 and 3 and the curve of the averages in the chart. First, it should be noted, *there was no diurnal rhythm.* The

5. Ortiz Picón, J. M.: Ztschr. f. Zellforsch. u. mikr. Anat. **23**:779, 1933.
6. Cooper, Z. K., and Franklin, H. C.: Anat. Rec. **78**:1, 1940.
7. Carleton, A.: J. Anat. **68**:251, 1934.

minor variations between averages when these are compared in every possible combination are without any statistical significance. The average mitotic activity at 8 a. m., 4 p. m., 12 p. m. and 4 a. m. is almost identical. Individual values of mitotic activity for carcinoma show much less variation than those for epidermis. Second, *the mitotic activity in the carcinoma was no greater than the maximum mitotic activity in an equal volume of normal epidermis.* At 8 a. m. and 12 m. there was no significant difference in average number of mitoses between carcinoma and normal epidermis. During the remainder of the twenty-four hour period, as mitotic activity remained constant in the tumors and waned and waxed in the normal epidermis, the differences were statistically significant.

COMMENT

The application of these results to neoplasms in general is contingent on the demonstration of similar results in other cancers, at different ages and in various species. The observations of Dublin, Gregg and Broders [1] are inadequate to furnish support. Therefore, this discussion must be predicated on the assumption that the results are generally true.

A neoplasm is an autonomous or independent growth. This implies that normal tissue growth is dependent. The demonstration of periodicity of mitotic activity is evidence of control of growth. Periodicity being different for each of three organs studied in the rat,[3] it is probable that the factors directly concerned in the daily regulation of growth are not central, not within the cell, but in the organ. It must be stressed that attention is here directed toward the factors which daily regulate growth. Heredity, hormones, nutrition, the nervous system, all play their part. However their manifold influence may be integrated, whatever their role may be in affecting mitotic activity, the organ is apparently the unit through which these factors may act on the cell.

A normal cell as the constituent of an organ has two types of activities: vegetative and functional. Among the vegetative activities is cell division. The functional activities are the result of differentiation and organization of cells. It has long been supposed that there is a partition of labor in the cell. It may be hypothesized that as a cell differentiates to become part of an organ it develops a component specifically affected by the functional stimuli peculiar to the organ of which it is a part—a component responsible for the shift between vegetative and functional activity in the cell. Thus, the cells of each organ may be supposed to have a characteristic component. In the submaxillary salivary gland it would be affected by the influences stimulating the secretion of saliva; in the kidney, by influences stimulating the secretion of urine. When affected, it would shift cell activity from vegetative to functional.

On the basis of this hypothesis, an explanation may be offered for the constant mitotic activity observed in these experimental tumors. The component of the normal cell, affected by the influences leading to function of the organ of which that cell is a part, in the tumor cell has been partially or completely altered or lost by dedifferentiation. There is thus no, or no regular, shifting of cell activity from the vegetative to the functional. The tumor cell behaves at all times as the normal cell does only during the period of least functional activity. Therefore, the tumor exhibits constant mitotic activity.

SUMMARY

The rate and the periodicity of mitotic activity were studied in well differentiated squamous cell carcinoma produced experimentally in male CBA mice by the application of a 0.3 per cent solution of methylcholanthrene in benzene. Normal epidermis of the same mice served as the control.

Mitotic activity in the cancers showed no diurnal rhythm but remained at a practically constant level throughout the day and the night. Mitotic activity in the normal epidermis exhibited the characteristic diurnal rhythm.

The rate of mitotic activity in carcinoma was no greater than the maximum rate in an equal volume of normal epidermis.

TUBERCULOID REACTION IN OVARIAN DYSGERMINOMA

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Dysgerminoma of the ovary is a relatively uncommon tumor. The reported incidence varies from 3.1 per cent (Klaften [1]) to 6.2 per cent (Sailer [2]) of all primary ovarian cancers. The term "disgerminoma" was introduced by Robert Meyer.[3] The inconsistency in the spelling, with the prefixes "dys" and "dis" used with almost equal frequency, is confusing. The prefix "dys" seems preferable, implying "deranged"; thus "dysgerminoma" would literally indicate a growth of deranged germinal cells, which is in accordance with the views of Meyer, whose theory as to the origin of the tumor from undifferentiated embryonic sex cells is rather generally accepted. The tumor has been known by several different names, the more common being "large cell carcinoma of the ovary" and "seminoma ovarii." The testicular counterpart is the seminoma testis (embryonic carcinoma), which histologically bears a striking resemblance to the ovarian dysgerminoma. The clinicopathologic features of the latter have been well described by Novak [4] and Seegar.[5]

Histologically, the dysgerminoma is one of the most constant and characteristic of ovarian tumors. It is composed of compact, fairly small, uniform islands of tumor cells surrounded by a loosely constructed fibrous stroma, giving it a characteristic reticulated structural pattern (*A* in figure). In areas, however, the tumor may grow as broad amorphous masses or in elongated, infiltrative columns; this is especially true of the secondary growths. The tumor cells are large, round and undifferentiated, containing large spherical vesicular nuclei that are often hyperchromatic. The cytoplasm is relatively sparse, stains poorly and is often hydropic. Within the fibrous network sur-

From the Butler County Memorial Hospital, Butler, Pa.

1. Klaften, E.: Arch. f. Gynäk. **158**:544, 1934.
2. Sailer, S.: Am. J. Cancer **38**:473, 1940.
3. Meyer, R.: Am. J. Obst. & Gynec. **22**:697, 1931.
4. Novak, E.: Gynecological and Obstetrical Pathology, ed. 1, Philadelphia, W. B. Saunders Company, 1940, p. 336.
5. Seegar, G. E.: Arch. Surg. **37**:697, 1938.

rounding the tumor islands are varying numbers of lymphoid cells and at times large irregular cells resembling mononuclear phagocytes of the epithelioid type (*B* in figure). Multinucleated giant cell forms are occasionally encountered.

Interpretations of the nature of the stromal reaction have been somewhat controversial. Novak and Gray [6] considered the epithelioid and giant cells as degenerated tumor cells. To Schiller [7] they are not constituents of the tumor but a product of the stromal reaction. He postulated that the tumor produces a lipoid substance similar to that of the tubercle bacillus, which stimulates a tuberculoid stromal reaction. He demonstrated lipoids in the tumor, a finding which has been confirmed by Greenblatt and Pund.[8] Schiller's interpretation would be completely substantiated if the lipoid could be shown by chemical and biologic reactions to be related to the lipoid of the tubercle bacillus (Sabin, Doan and Forkner [9]).

The origin of the lymphoid cells is not clear, although they are generally considered to be lymphocytic. Seegar [5] suggested that they might be "extruded polar bodies."

Occasionally the stromal reaction in dysgerminoma has assumed a tubercle-like arrangement, actually mistaken for tuberculosis. Kermauner [10] reported five granulosa cell tumors accompanied by tuberculosis, which Schiller [7] in a subsequent review reclassified as dysgerminoma. Schiller concluded that the stromal reaction was not tuberculous.

Only a few isolated cases exhibiting the tuberculoid reaction have been reported in the English literature (Sailer [2]—cases 1 and 2; Greenblatt and Pund [8]—photomicrograph in case 5; Novak and Gray [6]—case 12 [11]). At times a granulomatous reaction of a foreign body type has been observed.[12] The 2 cases reported here demonstrate the tuberculoid reaction of dysgerminoma. In case 1 the diagnosis of tuberculous salpingitis complicating the dysgerminoma was seriously considered for

6. Novak, E., and Gray, L. A.: Am. J. Obst. & Gynec. **35**:925, 1938.

7. Schiller, W.: Arch. f. Gynäk. **156**:513, 1934.

8. Greenblatt, R. B., and Pund, E. R.: Am. J. Obst. & Gynec. **35**:667, 1938.

9. Sabin, F. R.; Doan, C. A., and Forkner, C. E.: Am. Rev. Tuberc. **21**: 290, 1930.

10. Kermauner, cited by Schiller.[7]

11. The findings in this case were stated to have only a superficial resemblance to tuberculosis.

12. Dockerty, M. B., and MacCarty, W. C.: Am. J. Obst. & Gynec. **37**:878, 1939.

a time. Subsequent studies failed to demonstrate the organism. Twelve days later case 2, presenting a similar complicating picture, strengthened the suspicion that the tuberculoid reaction was secondary to the tumor.

REPORT OF CASES

CASE 1.—A 22 year old white woman, married, was admitted to the Butler County Memorial Hospital Jan. 7, 1942, complaining of intermittent abdominal pain of three months' duration, which radiated to the right hip and thigh. The previous medical history was irrelevant. The menses had been normal except for the preceding three periods, which were scanty.

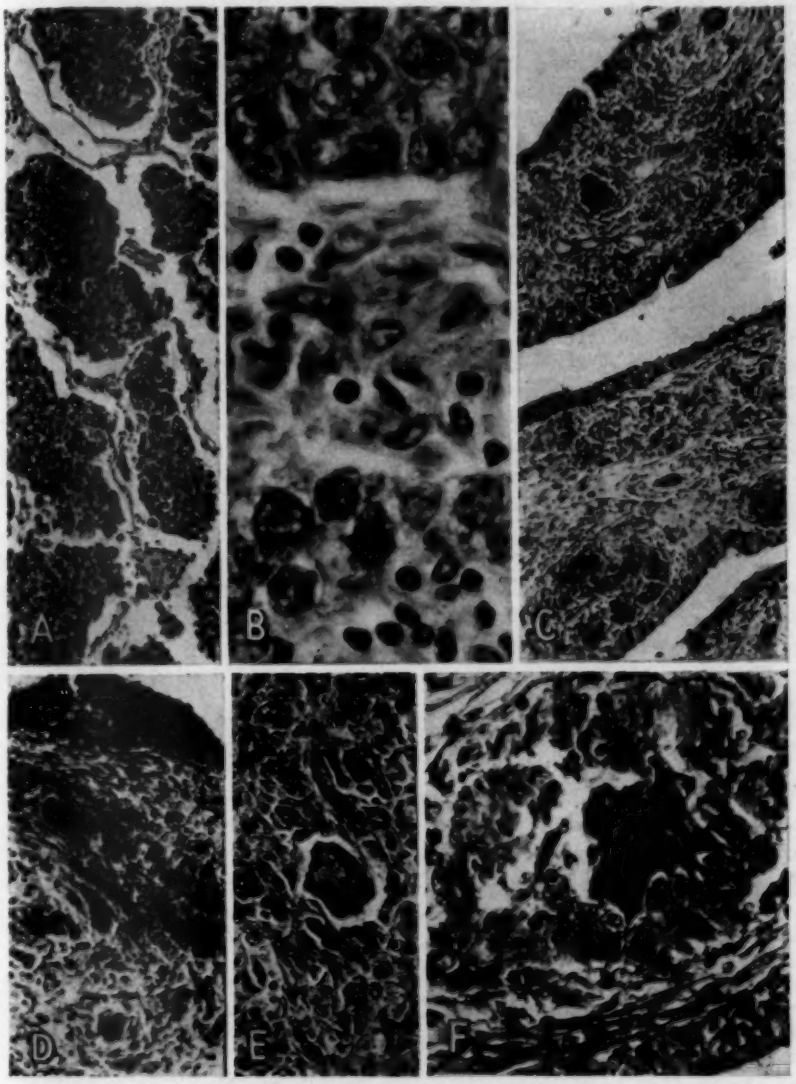
There was a large firm rounded mass in the lower portion of the abdomen. No additional abnormalities were noted.

At operation (Dr. C. A. Robb) the mass completely replaced the right ovary and had extensively infiltrated the right fallopian tube and broad ligament; metastases were noted in many of the regional and paravertebral lymph nodes and the opposite ovary. The adnexae uteri on both sides and the tumor mass were resected with difficulty.

The postoperative course was uneventful except for several days of intermittent fever. There were no signs of pulmonary disease on roentgen examination after operation. A course of treatment with high voltage roentgen rays was given postoperatively, during which the palpable tumor nodules rapidly regressed until they were no longer detectable. At the time of writing, approximately one year after operation, the patient presents no evidence of recurrence and is free of complaints.

Operative Specimen.—This consisted of a large rounded tumor mass, an ovary and a thickened, distorted broad ligament. The tumor mass measured 14 by 11 by 8 cm. Its surface was irregularly nodular and was covered with a thin fibrous capsule, which in areas was ragged and hemorrhagic. The tumor tissue was light yellowish gray and moderately firm. On section it was irregularly and coarsely lobulated by fibrous septums; the tumor tissue was compact, glistening and translucent. A few scattered small areas of softening and degeneration were noted. Along one edge of the mass was a distorted fallopian tube, the wall of which was irregularly nodular and thickened by infiltrating tumor. The opposite ovary was of normal size; protruding from its capsule at one point was a flattened pea size yellowish pink papular nodule which extended but a short distance into the underlying stroma. It was solid and resembled tumor. The broad ligament was diffusely infiltrated by tumor.

Microscopically, the tumor was a typical dysgerminoma. The stromal reaction was pronounced in certain areas. The outermost portion of the fallopian tube was extensively infiltrated with tumor which at times extended to the base of the plicae; rarely the tumor infiltrated the stroma of the plicae. Associated with the tumor, but often quite remote from it, was a granulomatous inflammatory reaction with formation of numerous miliary lesions strikingly similar to tubercles (*C* in figure). These were frequently situated within plicae uninvolved by tumor. Many such lesions were noted in the intimal layer of larger veins at some distance from the tumor (*D* in figure). The small nodule in the left ovary consisted of infiltrative columns of dysgerminoma cells associated with a pronounced tuberculoid reaction of the stroma.



EXPLANATION OF PLATE

A, typical structural pattern of dysgerminoma in case 1. × 120.

B, epithelioid and lymphocytic infiltration of the stroma between islands of tumor cells in case 1. × 700.

C, pseudotubercles in the plicae of a fallopian tube in case 1. × 76.

D, polypoid granulomatous reaction of the intima of a vein in the wall of the involved fallopian tube in case 1. The tumor has not involved the vessel. × 185.

E, dysgerminoma with pronounced stromal reaction in case 2. × 153.

F, pseudotubercle of the fallopian tube in case 1. × 375.

CASE 2.—A 25 year old white woman, married, entered Butler County Memorial Hospital Jan. 19, 1942, complaining of pain in the lower part of the abdomen of one week's duration. The last menstrual period occurred in the preceding July, and she considered herself to be approximately six months pregnant. She had had no previous pregnancies. The remainder of the history was irrelevant. Physical findings were normal except for a large rounded mass in the lower part of the abdomen, which extended upward to the level of the umbilicus.

At operation (Dr. C. A. Robb) two large solid rounded tumor masses replaced both ovaries. The capsule of the tumor on the left was extensively adherent to the posterior surface of the uterus, the omentum, loops of intestines and the parietal peritoneum. The uterus was of normal size (nulliparous). Neither metastasis nor local infiltration was apparent. The tumor masses, the uterus and both tubes were removed. Convalescence was uneventful except for several days of intermittent fever. Roentgenograms of the chest taken postoperatively revealed normal-appearing heart and lungs. The patient received a course of treatment with high voltage roentgen rays postoperatively. At the time of writing, approximately one year later, there is no evidence of recurrence, and the patient has no complaints.

Operative Specimen.—This consisted of two large tumor masses, the uterus and torn portions of the fallopian tubes. The tumors were approximately of equal size, measuring individually 16 by 12 by 7 cm. Their combined weight was 1,370 Gm. They were covered by thin fibrous capsules; in areas they were hemorrhagic and covered by adhesions. The masses were moderately firm, yellowish gray and nodular. On section the cut surface bulged slightly, was compact and glistening and showed areas of hemorrhage and necrosis. Variously sized lobules were formed by delicate fibrous septums.

The uterus was small, with its serosa thickened and covered with torn adhesions and portions of the adnexae.

The tubes were slightly dilated and distorted by adhesions.

Microscopically, the tumor was typical dysgerminoma, with a pronounced stromal reaction, containing lymphocytes, epithelioid cells and scattered giant cells (*E* in figure). The giant cells were frequently of the Langhans type; rarely tubercle-like nodules were observed. There was no extension of the tumor to the fallopian tubes or to the uterus. The sections of tubes revealed moderate infiltration by chronic inflammatory cells but were in no manner suggestive of tuberculosis.

COMMENT

The stromal reaction in the 2 cases reported was unusual and pronounced although fundamentally similar to that of typical dysgerminoma. There was an unusually pronounced epithelioid cell infiltration, accompanied by a profuse lymphoid reaction. The tuberculoid reaction in case 2 resembled tuberculous granulation tissue without tubercles and was so intimately associated with the tumor that it would scarcely be misinterpreted as tuberculous. The tuberculoid reaction was more pronounced in the infiltrative secondary lesions of case 1 and at times occurred in the advancing borders, not intimately associated with the tumor cells.

Consideration of the following points has led to the conclusion that the epithelioid and the giant cells were inflammatory rather than neoplastic.

Morphology (phloxine–methylene blue stain).—The epithelioid cells showed considerable variation in size and contour in contrast with the tumor cells, which were notably uniform in size and shape. The relative proportion of cytoplasm to nucleus was much greater in the epithelioid than in the tumor cells. In the epithelioid cells, the cytoplasm was constantly acidophilic, and the nuclei were hypochromatic and vesicular. In the tumor cells, the cytoplasm was neutrophilic or lightly basophilic, and the nuclei were larger and made up of coarser chromatin masses.

The giant cells in these 2 cases were distinctive and characteristic, often containing a dozen or more nuclei. The cytoplasm was particularly abundant and retained the acidophilic staining reaction of the individual epithelioid cells. Morphologically, the giant cells were often indistinguishable from the Langhans giant cells of tuberculosis (*F* in figure). Epithelioid cells in the process of fusion could be seen frequently. In contrast, the tumor cells sometimes contained two or more nuclei, and apparent fusion with adjacent cells was noted, but they never formed giant cells of the Langhans type. Occasionally, discrete tumor cells were seen in the stroma adjacent to the epithelioid cells, from which they could be readily differentiated.

Mitotic Figures.—An exhaustive oil immersion study failed to reveal a single mitotic figure in the epithelioid cells, although large numbers of mitotic figures were present in the tumor cells.

Associated Cells.—Lymphoid cells within the stroma in previously reported cases of dysgerminoma were generally interpreted as true lymphocytes. In these 2 cases the lymphoid cells were numerous and were accompanied in smaller numbers by plasma cells and eosinophils. The latter two cell types are known to be inflammatory, and their presence strengthens the belief that the other stromal elements are a part of an inflammatory reaction. The presence of plasma cells and occasional eosinophils was previously observed by Spielman and Morton.[13]

Tubercle Formation.—The pseudotubercles with epithelioid and giant cells, occurring in the stroma, were usually associated with peripheral fibrosis. This often occurred at some distance from the tumor. In the cellular islands of neoplastic tissue, where connective tissue was scanty or absent, pseudotubercles were not found. They were present in the intima of veins at some distance from the tumor, which was interpreted as evidence that they were small foreign body granulomas stimulated by

13. Spielman, F., and Morton, F. L.: Am. J. Obst. & Gynec. **36**:665, 1938.

some product of the tumor. The presence of a lipoid substance in dysgerminoma suggested that this substance might be the foreign body about which the reaction occurred. The interpretation of Schiller seems to be the best explanation for the presence of pseudotubercles in dysgerminoma.

SUMMARY

Two cases of ovarian dysgerminoma accompanied by an unusual tuberculoid stromal reaction are reported. The epithelioid and giant cells are most likely inflammatory in character rather than neoplastic.

A POSTMORTEM STUDY OF THE RENAL PELVIS IN RELATION TO HYPERTENSION

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AND
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DETROIT

Interest has recently been aroused in the relation between the anatomy of the renal pelvis and hypertension. Campbell [1] in 1941 reviewed 137 cases of infravesicular obstruction, in 19 of which the obstruction was associated with hypertension. He suggested that hypertension should occur more readily in those cases in which the pelvis is in the intrarenal position.

Ravich [2] went further, saying that in the human species a minority retain throughout life a fetal type of kidney on one or both sides, in which the pelvis is in the intrarenal position. He stated further that the intrarenal pelvis tends to persist in certain families throughout life and that in this group hypertension frequently develops. He explained that the mechanism consists of two factors; the first factor is a pelvis in the intrarenal position, and the second, a back pressure developing in the pelvis because of some superimposed condition. The distended pelvis in a narrow hilus was thought to exert pressure on the large renal arteries, producing renal ischemia and thereby hypertension. However, if the same conditions obtain in a kidney with the pelvis in the extrarenal position, on account of the wide hilus a major portion of the pelvis is not closely surrounded by renal parenchyma, and severe dilatation can occur without appreciable pressure on the renal vessels. Ravich supported this theory by a review of 200 intravenous urograms. He was "amazed at discovering the regularity with which hypertension was accompanied by disease in the intrarenal type of kidney pelvis. On the other hand, because of the cushioning effect inherent in the extrarenal type, essential hypertension in such protected kidneys appeared extremely unlikely even though the existing pathologic changes were of considerable degree."

More recently Sarnoff [3] examined the pyelograms in a series of cases of hypertension and concluded that there was no significant relation

From the Departments of Pathology and Medicine of Wayne University College of Medicine and the Department of Pathology of Receiving Hospital.

1. Campbell, E. W.: J. Urol. **45**:70, 1941.
2. Ravich, A.: J. Urol. **46**:641, 1941.
3. Sarnoff, S. J.: J. Urol. **47**:769, 1942.

between the type of renal pelvis and hypertension as judged by clinical urography.

The purpose of our study was to determine by the use of postmortem urography and statistical analysis whether there is any significant correlation between the position of the renal pelvis and hypertension.

METHOD AND MATERIAL

A radiopaque mass was injected in a retrograde manner into the ureter and renal pelvis under controlled pressure. A permanent record was obtained by making a roentgenogram of the injected specimen. The injection mass and the apparatus were similar to those used by Schlesinger [4] for the study of hearts.

With a wax pencil, the hilar rim was then outlined on the roentgenogram by comparison with the gross specimen. Actual measurement was employed when the hilar rim was not plainly discernible on the roentgen plate. Sections were then taken for microscopic study.

In this manner it was possible to record exactly the position of the renal pelvis, the hilar rim, the contour and the size of the kidney, the proximity of the parenchyma to the pelvis and the calices, or other anatomic features of the specimen.

The series consisted of 76 cases which were, in the main, consecutive. No selection was attempted, and the group is representative of cases which come to necropsy in the hospital.

By measurement of the pyelogram it is possible to locate exactly the pelvis in relation to the parenchyma of the kidney. This was done in the following manner: A triangle was drawn between the ureteropelvic junction and the bottom portion of the superior and that of the inferior major calix. The center of such a triangle was found at the intersection of two lines drawn, one from each base angle to the midpoint of the opposite side. This point, while not the true center of the pelvis, serves as a point of reference by which the type of pelvis in any individual specimen can be determined.

This method being used, an intrarenal pelvis is defined as a pelvis in which the reference point falls lateral to or within the hilar rim (*A* in figure). By definition an extrarenal pelvis is one in which the point of reference falls medial to the hilar rim (*B* in figure).

Cases in which the diastolic pressure was 90 mm. or above or in which the microscopic sections of the kidney revealed arteriosclerosis were considered to show hypertension. The series was then arranged in four groups depending on the position of the pelvis and on the presence or absence of hypertension. The results of this grouping are seen in the accompanying table.

It has been demonstrated by several investigators [5] that ischemia of only one kidney causes hypertension. Since such is the case, it seemed only fair that for the purpose of mathematical analysis all cases in which one kidney was of the extrarenal and the opposite of the intrarenal type should be included in the intrarenal group. In the table 14 such cases are placed in the intrarenal division. These account for 18.4 per cent of the total 76 pairs of kidneys.

A survey of our tabulated data does not lend support to the theory proposed by Ravich. On inspection of the table it may be seen that the pelvic types were equally represented, there being 38 cases of each.

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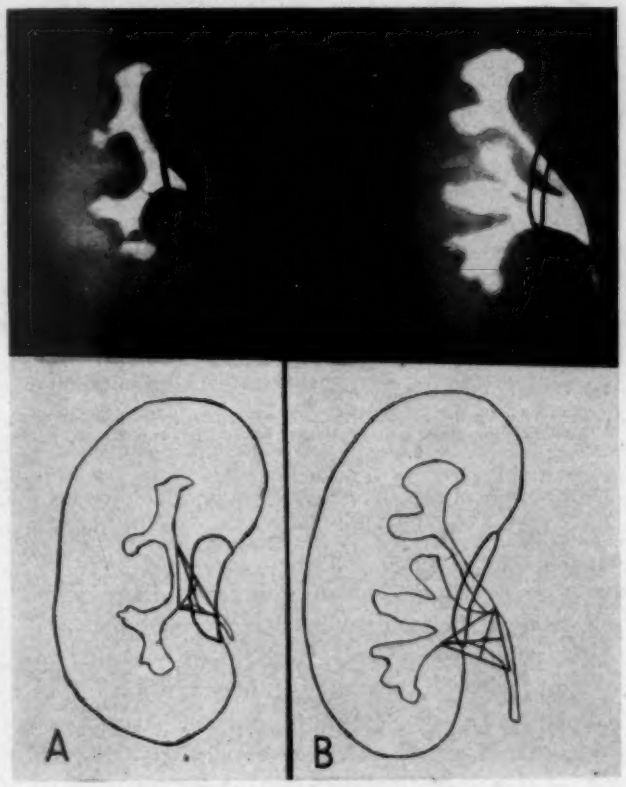
4. Schlesinger, M. J.: Am. Heart J. **15**:528, 1938.

5. Goldblatt, H.: Am. J. Clin. Path. **10**:40, 1940. Wilson, C., and Byrom, F. B.: Lancet **1**:136, 1939. Page, I. H.: J. A. M. A. **113**:2046, 1939.

The cases in which hypertension was present were about equally divided, 14 in the intrarenal and 15 in the extrarenal group. These 29 cases in which hypertension was present make up about 38 per cent of the total number of cases studied. Likewise, the cases in which no hypertension was observed were fairly equally distributed between the two aforementioned groups. When these data were subjected to the chi square test, the value for chi square was found to be 0.0552 and the



Roentgenograms of kidneys, with tracings to illustrate the location of the reference point in a case of intrarenal pelvis (*A*) and in a case of extrarenal pelvis (*B*).

corresponding probability 0.8132. This supports the contention that there is no relation between the anatomic type of pelvis and the development of hypertension because with a probability value of 0.8132 there is an eight to ten chance that the slight difference in number of cases in each group is due to errors in sampling alone.

The fact that kidneys as found in the human species are frequently rotated on the sagittal axis so that the pelves are directed anteriorly or

posteriorly was exhaustively investigated by Kelly and Burnam.[6] These investigators found that the topography of the renal vessels was greatly affected by the degree of rotation. In the more severe degrees of rotation the majority of the vessels tend to follow a curved course over the hilar rim before entering the parenchyma. It seemed theoretically possible that ischemia might be more readily produced when pressure is exerted on the severely angulated vessels, bordered as they are on one side by unyielding renal substance.

Our series was regrouped and statistically analyzed for the relation between rotation and hypertension. No significant degree of correlation was found. The fact that fetal lobulation was frequently associated with the immature type of kidney has been mentioned.[2] We therefore studied in a like manner the relation between fetal lobulation and hypertension and could demonstrate no significant correlation.

*Grouping of Cases in Series**

Type of Pelvis	Hypertension	No Hypertension	Total
One or both intrarenal	14	24	38
Both extrarenal	15	23	38
Total	29	47	76

Chi square = 0.0552 Probability = 0.8132

* Pearl, R.: Medical Biometrics and Statistics, ed. 2, Philadelphia, W. B. Saunders Company, 1940.

During the course of this investigation it became obvious that rotation of the kidney makes intravenous urography an unreliable method of determining the type of renal pelvis.

We therefore conclude that in this series there is no statistical evidence to support the contention that hypertension and intrarenal position of the pelvis are related. Nor do we feel that the degree of rotation or fetal lobulation is related to hypertension. It is interesting to note that the conclusions drawn by Sarnoff,[3] based on antemortem pyelography, agree essentially with the conclusions at which we arrived by the study of postmortem urographs.

SUMMARY

A method for exactly determining the position of the pelvis relative to the parenchyma of the kidney is described. This method was applied in a representative series of routine autopsies. The results were statistically analyzed to determine any significant correlation between the intrarenal pelvis and hypertension. No such correlation could be demonstrated. Likewise, no significant correlation was found between hypertension and rotation or between hypertension and fetal lobulation.

6. Kelly, H. A., and Burnam, C. F.: Diseases of the Kidneys, Ureters and Bladder, with Special Reference to the Diseases in Women, New York, D. Appleton and Company, 1914; ed. 2, 1922.

TYROSINE POISONING IN RATS

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Tyrosine occupies a key position in the production of epinephrine, melanin, thyroxin and posterior pituitary hormone. As a precursor of tyramine it has been related to the causation of "pale hypertension," while as the chief component of melanin it has been suspected as a factor responsible for the incidence of primary cancer of the liver among the colored races. Umber,[1] as well as Weiss,[2] commented on the urinary excretion of tyrosine in hepatic diseases and its relation to the frequently coexisting pancreatitis, whereas Baer and Blum [3] and Neubauer [4] stated that tyrosine is one of the four amino acids (leucine, isoleucine, tyrosine and phenylalanine) responsible for the formation of ketone bodies. Bickel [5] noted the aglycogenoplastic properties of tyrosine, as it prevents the accumulation of glycogen in the liver, and called attention to the importance of this amino acid in the dietary management of diabetes.

Litzka [6] mentioned that tyrosine fluoride causes a reduction of the blood sugar in patients with diabetes or exophthalmic goiter, and Abelin [7] recommended, on the basis of animal experiments, the administration of tyrosine to patients with hyperthyroidism as a detoxicant of the thyroid hormone. A similar suggestion was made by Medes,[8] who warned, however, against the use of excessive doses of this substance, as she observed directly following such medication, an increase in basal metabolism, an effect which was also noted by Abelin.

Concerning the possible toxic effects of such a treatment, Abelin stated that tyrosine is rapidly metabolized in the animal organism and

From the Warner Institute for Therapeutic Research.

1. Umber, F.: Erkrankungen der Leber, der Gallenwege und des Pankreas, in Mohr, L., and Staehelin, R.: Handbuch der inneren Medizin, Berlin, Julius Springer, 1926, vol. 3, pt. 2, p. 1.
2. Weiss, M.: Klin. Wchnschr. **15**:521, 1936.
3. Baer, J., and Blum, L.: Arch. f. exper. Path. u. Pharmakol. **56**:92, 1907.
4. Neubauer, O.: Intermediärer Eiweissstoffwechsel, in Bethe, A.; von Bergmann, G.; Embden, G., and Ellinger, A.: Handbuch der normalen und pathologischen Physiologie, Berlin, Julius Springer, 1928, vol. 5, p. 851.
5. Bickel: Klin. Wchnschr. **18**:178, 1939.
6. Litzka, G.: Klin. Wchnschr. **15**:1568, 1936.
7. Abelin, I.: Klin. Wchnschr. **14**:1777, 1935.
8. Medes, G.: Biochem. J. **26**:917, 1932.

can be given in large amounts without any danger. This claim is apparently confirmed by the observations of Abderhalden and co-workers,[9] who took within one day from 50 to 150 Gm. of tyrosine without any apparent ill effect. Similarly, Medes administered to normal persons from 5 to 10 Gm. and occasionally as much as 50 Gm. of this amino acid without their experiencing untoward reactions. Newburgh and Marsh,[10] on the other hand, when working with dogs and rabbits noted that tyrosine given in doses of 2 Gm. per kilogram of body weight causes renal injury. Lillie [11] recorded the occurrence of minor tubular degenerations of the kidney, exudative blepharitis and red, edematous swelling of the extremities in rats kept for as long as twenty days on a basal diet containing as much as 20 per cent tyrosine (Sullivan, Hess and Sebrell [12]). Liver, small intestine, spleen, skeletal muscle and eye, also studied, were without abnormalities.

The observations to be reported were made during investigations on the role of tyrosine in the causation of hypertension, conducted by one of us (Martin [13]), and represent a continuation of previous experimental studies in cardiovascular pathology (Hueper [14]).

EXPERIMENTAL OBSERVATIONS

Black rats, 5 weeks old, were placed on a basal diet consisting of sucrose 57 parts, casein 18 parts, cod liver oil 2 parts, butter fat 9 parts and salts 4 parts, with the addition of thiamine hydrochloride 5 mg., riboflavin 10 mg., pyridoxine 5 mg., calcium pantothenate 100 mg., inositol 100 mg., paraaminobenzoic acid 100 mg., nicotinic acid 100 mg. and choline 200 mg., and the replacement of 10 additional parts of sucrose usually present in the diet by an equivalent amount of l-tyrosine (Martin). The l-tyrosine was supplied by Dr. Arnold H. Johnson, Research Laboratories, Sealtest, Inc., Baltimore. With the tyrosine contained in the casein, the diet thus contained 11.17 parts of tyrosine. Within one week the animals had crusted eyelids, opacities and ulcers of the cornea and swollen, red legs and feet. The majority of the rats died within three weeks, while a few survived almost five weeks (thirty-three days). Of the large number of rats thus treated and used for measurements of blood pressure and chemical studies of the blood, 83 were available for pathologic investigations. Forty-two of these rats died spontaneously, and 41 were killed for the purposes just mentioned. Twenty-five rats survived for seven to ten days, 50 for eleven to nineteen days and 8 for twenty to thirty-three days.

The autopsy showed the meningeal vessels engorged and, in some animals, surrounded by large hemorrhagic areas. The lungs were congested and frequently

9. Aberhalden, E.: Ztschr. f. physiol. Chem. **77**:454, 1912

10. Newburgh, L. H., and Marsh, P. L.: Arch. Int. Med. **36**:682, 1925.

11. Lillie, R. D.: Pub. Health Rep. **47**:83, 1932.

12. Sullivan, M. X.; Hess, W. C., and Sebrell, W. H.: Pub. Health Rep. **47**:75, 1932.

13. Martin, G. J.: Arch. Biochem., to be published.

14. Hueper, W. C.: Arch. Path. **34**:883, 1942.

contained hemorrhagic foci. The heart was contracted. The pancreatic tissue was embedded in an edematous matrix in an appreciable number of animals and was enlarged in mass in others. The liver and the spleen were grossly normal. The kidneys of a considerable number of rats showed a finely granular, light brownish surface. The testes were small, corresponding to the age of the animals.

The histologic examination of the various organs rendered the following results:

The meningeal vessels were often highly congested and not infrequently surrounded by extensive hemorrhagic areas. In some instances there existed considerable perivascular fibrosis. Similar changes were present along the vessels extending into the brain substance. The cerebral vessels were usually hyperemic also and often surrounded by small hemorrhages. The arterioles were contracted and exhibited, particularly in rats which survived for more than ten days, swelling and hyalinization of the vascular walls (fig. 1 *A*). Some of the vessels showing these changes were surrounded by glia cells and leukocytes; others, by an edematous zone. The brains of a few rats contained small necrotic foci with glial and leukocytic infiltrations. The ganglion cells not infrequently exhibited degenerative changes, such as balloon-like swelling and vacuolation, while in a rat which survived for twenty days a group of calcified ganglion cells was observed (fig. 1 *B*).

The anterior lobe of the hypophysis was congested in most instances and was composed of strands of eosinophilic cells surrounding groups of chromophobic cells. The median and posterior lobes were normal.

Many of the eyeballs examined showed mild to severe degenerative and inflammatory changes of the cornea. These extended rarely to the eyelids. In the mild conditions the corneal lesions consisted of edema and scanty infiltration by mononuclear cells and leukocytes and were most marked in the marginal portions. In more advanced conditions, involving the entire cornea, there was dense accumulation of leukocytes and lymphocytes, associated with invasion of capillary vessels. The epithelial lining was eroded, and the ulcerative defects were covered by a leukocytic exudate. The rest of the eyeball was intact.

The thyroid glands of a few animals were normal. The glands of the majority and particularly those of rats surviving for more than twenty days consisted of small follicles filled with thin, vacuolated colloid. There were, moreover, areas of solid follicles and follicles with a proliferated epithelial lining.

The lungs were usually congested, and many of them contained scattered hemorrhagic areas. The pulmonary arterioles of 8 rats exhibited thickened and often hyaline walls lined by a crowded endothelium arranged in palisade formation.

A small myocardial hemorrhage was found in a single rat, while a small focus of myocardial calcification was present in a second. The myocardial arterioles were thickened in 10 animals and were hyaline in some. The frequency of this change increased with the duration of the treatment.

The liver often showed some degree of congestion. The liver cells had in many instances a finely granular cytoplasm, while in some the liver exhibited a moderate degree of pericentral fatty degeneration with large vacuoles or contained scattered small hyaline necroses.

The spleen was in general normal. In a few instances there was general atrophy of the pulp, associated with scattered hyaline necroses.

The stomach and intestines showed no abnormality.

The pancreas was normal in 17 instances. In all others it showed more or less extensive lesions. The interstitial connective tissue was often highly edematous

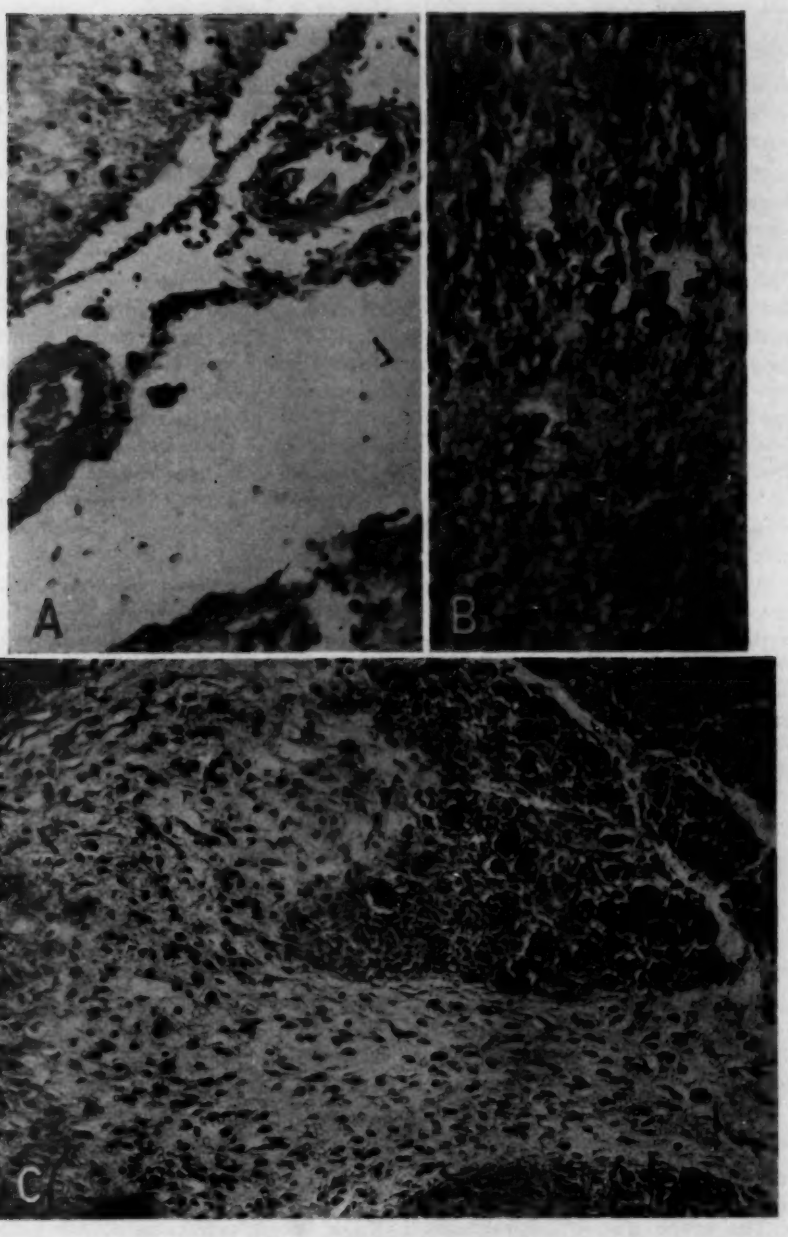

Fig. 1.—*A*, meningeal arterioles with polypous endothelial proliferations. *B*, small focus of necrosis in the cortex, with accumulation of glia cells, and pyknosis and calcification of ganglion cells. *C*, edema and fibroblastic proliferation in the interstitial tissue of the pancreas, with degeneration and necrosis of some pancreatic lobules.

and infiltrated to varying degrees by large mononuclear cells and eosinophilic leukocytes (fig. 1 *C*). The pancreatic lobules which were embedded in this matrix sometimes contained only a few necrotic foci, usually infiltrated and surrounded by eosinophilic leukocytes. In other instances there was evidence of a prolonged and progressive degenerative process. The exocrinic cells of some irregularly shaped acini were small and free from zymogen granules. They possessed a faintly stained, vacuolated cytoplasm and indistinct outlines. Their nuclei were pale, irregular in shape and reduced in size. In other lobules this process had progressed further. The acini were necrotic or had collapsed after the lysis of the cells, leaving small cysts. The increased intralobular connective tissue frequently formed nodules of large spindle-shaped cells, infiltrated by lymphocytes and eosinophilic leukocytes (fig. 2 *A*). These were also found in many instances within the necrotic exocrinic glandular foci. The islets were usually normal in appearance and number during this stage, or they showed moderate to marked swelling, characterized by the appearance of strands of large vesicular cells in loose gyriform formations. In still older lesions much of the pancreatic tissue had been replaced by hyaline necroses and fibroblastic tissue containing usually a varying number of mononuclear cells and eosinophilic leukocytes. The general pattern was that of a multinodular granuloma, throughout which small remnants of the pancreatic tissue were scattered. There was marked reduction in the number of islets, which had been engulfed in the general cirrhosis (fig. 2 *B*). However, isolated islets surrounded by large areas of fibrous tissue were found occasionally. The blood vessels and the ducts were in general normal. Moderately dilated ducts occurred only infrequently.

In the adrenal glands, the medullary sinuses were often engorged. A diffuse hemorrhage into the medulla and the cortex was present in 1 case.

The kidneys in the majority of instances showed hyaline nephrotic changes. In approximatelly 30 per cent of the cases they showed, moreover, focal necrotizing lesions, involving the cortex as well as the medulla, often affecting wedge-shaped zones. The mildest reactions of this type consisted in accumulations of leukocytes in the tubular lumens, with moderate leukocytic infiltration in the surrounding tissue, and cystic distention of tubules, with atrophic, metachromatic, dark-stained cells in the corresponding cortical areas. The cystically distended tubules contained occasionally an albuminous pink or brown matter and were surrounded by thickened and cellular interstitial tissue. In more advanced lesions there were central hyaline necroses surrounded by leukocytes and fibroblasts. The pink-stained necrotic centers consisted of a peculiarly filamentary matter arranged in radiating fashion (fig. 2 *C*). In several instances there was diffuse granulomatous involvement of the tip portion of the pyramids. The pelvic epithelial lining was then eroded and covered by thick leukocytic exudate which also filled the renal pelvis. The glomeruli were usually intact. The arterioles in an appreciable percentage showed thickened and sometimes hyaline walls and occasionally proliferation of the endothelial cells.

In the testes, the spermatogenic epithelium was in general of the immature type found normally in animals of this age. However, in 8 rats which had been exposed for more than ten days to the tyrosine diet, definite evidence of epithelial atrophy and of spermatid giant cell formation was obtained.

The marrow of the sternum was frequently highly congested and in an appreciable number of instances contained large hemorrhages. The latter condition was associated in general with moderate to marked atrophy of the myeloid tissue. A few additional animals exhibited atrophic myeloid tissue with scattered hyaline necroses.

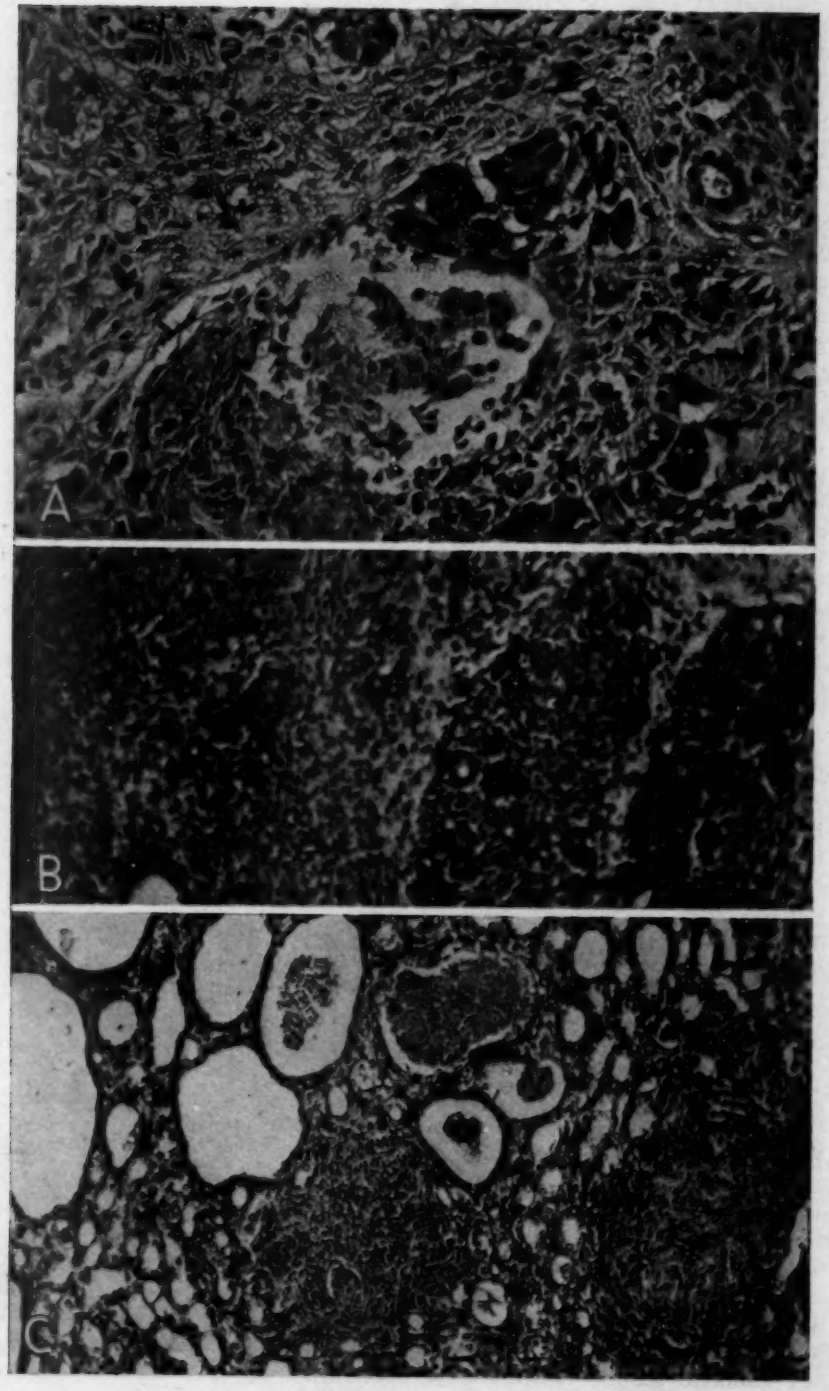

Figure 2

(See legend on opposite page)

COMMENT

The recorded observations, some of which confirm those previously reported by Lillie,[11] reveal that tyrosine poisoning as produced in rats is characterized by two main types of pathologic changes, those of degenerative and necrotizing nature, affecting the parenchyma of the pancreas, the eye, the kidney, the brain, the liver, the spleen, the testes and the marrow, and those of a circulatory-vascular genesis, represented by congestion, hemorrhages, edema, perivascular fibrosis and arteriolar medial degeneration, affecting the brain, the lungs, the heart, the adrenal glands, the kidneys and the marrow.

The most striking and extensive inflammatory, degenerative and cirrhotic lesions were found in the pancreas. The intact status of the pancreatic vessels, as well as the primary involvement of the exocrinic tissues of this organ, does not favor the possibility that these lesions are the result of spastic arteriolar ischemia of the pancreatic vessels elicited either by intrapancreatic transformation of tyrosine into tryamine or by excessive production of epinephrine under the influence of an abnormal supply of tyrosine (Holtz[15]). It is also not likely that these changes were caused by a specific antipancreatic action of the secretion of the anterior lobe of the pituitary gland, possibly stimulated by tyrosine, since the degenerative changes produced in the pancreas by prolonged treatment with anterior pituitary extract are restricted to the islets and cannot be elicited in rats, though elicited readily in dogs and less easily in cats and rabbits (Young[16]; Marks and Young[17]; Richardson[18]). It is even maintained that the repeated introduction of anterior pituitary extract into rats is followed by hypertrophy and multiplication of Langerhans' islands (Anselmino, Herold and Hoffmann[19]).

15. Holtz, P.: Arch. f. exper. Path. u. Pharmakol. **186**:684, 1937.

16. Young, F. G.: J. Physiol. **92**:15, 1938; Proc. Roy. Soc. Med. **31**:1305, 1938.

17. Marks, H. P., and Young, F. G.: J. Physiol. **92**:14, 1938; J. Endocrinol. **1**:470, 1939.

18. Richardson, K. C.: Proc. Roy. Soc., London, s.B **128**:153, 1940.

19. Anselmino, K. J.; Herold, L., and Hoffmann, F.: Klin. Wchnschr. **12**:1245, 1933.

Explanation of Figure 2

A, nodular fibrosis of the pancreas, with hypertrophy of the islets and interstitial infiltration by eosinophilic cells.

B, advanced hyaline fibrosis of the pancreas, with small remnants of exocrinic and endocrinic pancreatic tissue.

C, multiple necrotic foci with cystically distended renal tubules.

The presence of purulent keratitis in many of the rats suggested that tyrosine or one of its metabolites might have caused an imbalance of the vitamin metabolism, as such ocular manifestations are found with various deficiency states due to lack of factors of the vitamin B complex, and as pancreatic atrophy and fibrosis sometimes accompany thiamine avitaminosis (Eddy and Dalldorf [20]; Wolbach [21]; Kihn [22]). However, the absence of any additional signs of thiamine deficiency and the rapid development of the pancreatic lesions in the rats kept on a tyrosine diet militate against the action of an avitaminotic mechanism.

There remains the possibility that the degenerative and necrotizing processes in the pancreas were caused either by activation of the pancreatic proteolytic enzymes by the tyrosine or by direct toxic action of tyrosine, excreted perhaps through the exocrinic tissues, on these elements. In considering these possibilities it may be noted that diseases of the biliary system are often associated with tyrosinemia (Umber [1]; Weiss [2]; Jankelson, Segal and Aisner [23]) and that pancreatitis and diabetes occur often as the so-called "second disease" in these conditions (Terbrüggen [24]; Gruber [25]). While it is assumed by many investigators that these pancreatic complications are the result of penetration of bile into the pancreatic duct and the activation of the proteolytic enzymes ensuing therefrom and causing inflammation and destruction of the pancreatic tissue, this contention has not remained unchallenged (Westphal [26]). The present observations suggest that a different and intrapancreatic mechanism connected with the tyrosinemia may be operative. It is obvious that diabetes mellitus developing on the basis of tyrosine poisoning is secondary to pancreatitis.

The degenerative and necrotizing lesions found in the renal parenchyma and pelvis are evidently of an excretory and toxic character and are unrelated to the arteriolar hyaline changes observed in some of the kidneys. The wedge-shaped distribution of the tubular involvement and especially the epithelial erosion at the tips of the pyramids support

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20. Eddy, W. H., and Dalldorf, G.: The Avitaminoses, ed. 2, Baltimore, Williams & Wilkins Company, 1941.

21. Wolbach, S. B.: Growth and Development of the Child: III. Nutrition, New York, The Century Company, 1932.

22. Kihn, B.: Avitaminosen und verwandte Krankheitszustände, Berlin, Julius Springer, 1927.

23. Jankelson, I. R.; Segal, M. S., and Aisner, M.: Am. J. M. Sc. **193**:241, 1937.

24. Terbrüggen, A.: Klin. Wchnschr. **17**:161, 1937.

25. Gruber, G. B.: Pathologie der Bauchspeicheldrüse, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 5, pt. 2, p. 211.

26. Westphal, K.: München. med. Wchnschr. **2**:1553, 1936.

this interpretation. A toxic factor is apparently also responsible for the foci of degeneration found in the liver, the spleen and the testes of some of the rats. Circulatory disturbances seem to be, on the other hand, the main cause of the foci of necrosis and degeneration observed in the brain and in the marrow, as these exhibited a close relation to hemorrhages or vascular lesions.

The degenerative medial changes found in an appreciable number of arterioles of the brain, the heart, the lung and the kidneys, sometimes associated with endothelial proliferation, represent the second remarkable feature of tyrosine poisoning, as they were elicited in rats of an age at which spontaneous occurrence of such lesions is unknown. The mechanism active in their production is uncertain, however. It is possible, though not likely, that tyrosine or a metabolite may have exerted a direct toxic effect on the vascular walls. However, inasmuch as the rats studied showed hypertension symptomatically (Martin), it is more probable that a hypertensive mechanism is involved in this respect, as tyrosine may give rise to the vasopressor amine, tyramine, or may be used in the production of epinephrine or of a vasopressor hormone of the pituitary or the thyroid gland. Consideration must be given, moreover, to the possibility that a renal pressor agent may have contributed to any pressor effect, as the kidneys of many rats revealed extensive degenerative tubular and interstitial mononuclear and fibroblastic changes.

The observations made are important for various reasons. They suggest interrelations between disturbances in the tyrosine metabolism, diseases of the biliary tract and the development of pancreatitis and diabetes mellitus. They add to information concerning the relationship between the activity of vasopressor agents and arteriosclerosis and arteriolosclerosis. They represent, finally, a warning against the introduction of excessive amounts of this amino acid for therapeutic purposes, as may occur during the proposed treatment of thyrotoxicosis.

SUMMARY

Rats kept on a diet containing 10 per cent l-tyrosine show within one to two weeks purulent keratitis and swelling and redness of the feet and legs, and die within five weeks.

They show degenerative, necrotizing and fibrosing changes of the pancreas, affecting primarily the exocrinic tissue, but leading ultimately to extensive destruction of the exocrinic and endocrinic tissues.

In the kidneys additional degenerative and necrotizing lesions are found, which apparently have their genesis in the excretion of a toxin.

The arterioles of the brain, the heart, the lungs and the kidneys exhibit swelling and hyalinization of the media and occasionally pro-

liferation of the intima, associated with focal hemorrhages, necroses and glia cell accumulations in the brain.

The observations suggest causal relations between diseases of the biliary tract, pancreatitis, diabetes and disturbances of the tyrosine metabolism and represent a warning against prolonged administration of excessive amounts of tyrosine for therapeutic purposes.

It may be pointed out, however, that tyrosine represents a normal protein constituent for which there is a definite nutritional and metabolic need. Reactions of the type described do not occur ~~as long as~~ unless the amounts of tyrosine introduced exceed by a wide margin the physiologic need of the organism for this amino acid.

EFFECTS OF REPEATED INTRAVENOUS INJECTIONS OF LECITHIN IN RABBITS

THE RELATIONSHIPS TO LIPOID STORAGE DISEASES AND TO HEMOLYTIC ANEMIAS

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Although the glycerophosphatides have not been specifically implicated in any lipoid storage disease, Kimmelstiel and Laas [1] and Epstein and Lorenz [2] found them to be increased in the tissues in Niemann-Pick disease and in Gaucher's disease, in addition to the lipoids primarily involved (the phosphatides classified as sphingomyelins and the galactolipid kerasin, respectively). Storage phenomena similar to those in Niemann-Pick disease were observed experimentally by von Beumer and Gruber [3] and Ferraro [4] following repeated intravenous injections of sphingomyelin, and much the same results, by Tompkins,[5] following similar injections of the ether-insoluble fraction of beef brain (a mixture of sphingomyelins and galactolipids).

Sjövall [6] expressed the belief that the glycerophosphatide lecithin, on the other hand, is utilized too rapidly in the normal organism to permit of storage except on administration of overwhelming amounts. He based this opinion in part on his failure to obtain storage phenomena following intravenous injections of this phosphatide in acute experiments, and he was supported in his opinion by similar failures on the part of von Beumer and Gruber [3] and Ferraro [4] in control studies which they performed with the glycerophosphatides. He did, however, obtain transitory accumulations of lipoid-containing cells following subcutaneous and intraperitoneal injections of lecithin, and Tompkins [7] found that subcutaneous injections of the glycerophosphatides (from brains and

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This material was presented before the American Society for Experimental Pathology.

1. Kimmelstiel, P., and Laas, E.: Beitr. z. path. Anat. u. z. allg. Path. **93**:417, 1934.
2. Epstein, E., and Lorenz, K.: Ztschr. f. physiol. Chem. **192**:145, 1930.
3. von Beumer, H., and Gruber, G. B.: Jahrb. f. Kinderh. **146**:125, 1936.
4. Ferraro, A.: Arch. Path. **30**:731, 1940.
5. Tompkins, E. H.: Am. J. Physiol. **129**:481, 1940.
6. Sjövall, A.: Acta path. et microbiol. Scandinav. **12**:307, 1935.
7. Tompkins, E. H.: Bull. Johns Hopkins Hosp. **70**:55, 1942.

eggs), as well as of sphingomyelins, elicit local infiltrations of macrophages comparable to the specific cells of the lipoid storage diseases. In view of these differences between the findings following intravenous and subcutaneous administration of lecithin, an investigation of the effects of intravenous injections repeated over long periods, in contrast to the more acute experiment of Sjövall,[6] seemed indicated.

MATERIAL AND METHODS

Data concerning the purity of the lecithin injected, the dosages, the periods over which injections were given and the weights of the rabbits and of the organs

TABLE 1.—*Data on Duration of Experiments, Dosages, Weights and Hemopoietic Organs*

Rabbit AF Series No.	Form in Which Commercial Lecithin Was Given	Period Over Which Injections Were Given, Days	Dosages			Weight, Gm.*			Bone Marrow Activity	
			Total, Gm.	Maximum Daily Gm.	Maximum Daily Days Given	Rabbit	Spleen	Liver	Estimated	Ratio to Myeloid Elements Erythroid Elements
23	Untreated.....	5	2.88	1.00	1	4,120	1.8	152	++	1.14
24	Untreated.....	74	37.94	1.00	6	4,400	8.6	112	++++	0.37
26	Untreated.....	44	25.30	0.88	18	3,060	5.4	150	+++	0.79
35	With the following	67	22.65	0.50	28	3,300	7.4	92	++++	0.66
36	impurities extracted: water-soluble, acetone-soluble, ether-soluble	55	16.95	0.40	37	3,800	1.9	100	++++	0.91
25	With cephalin extracted in addition to substances referred to above	57	25.43	0.79	14	4,560	5.2	200	+++	1.00

* The averages of the corresponding weights from 9 normal control rabbits killed in the same manner as the experimental animals are as follows:

Rabbit 3,800 Gm. (2,980 - 4,500)
Spleen 2.4 Gm. (1.13 - 3.72)
Liver 94.6 Gm. (68 - 110)

Bone marrow
Estimated activity ++
Ratio (M/E) 1.34 (0.8-2.4)

are presented in table 1. Commercial egg lecithin (Digestive Ferments Company) was employed merely because it is easy to obtain in bulk in relatively pure form. It was used without further purification for three experiments (rabbits 23, 24 and 26). For two others (rabbits 35 and 36) it was treated according to the method of MacLean[8] for removal of water-soluble impurities, followed by several extractions of the acetone-soluble and ether-insoluble lipoids (i. e., cholesterol, fatty acids, lysolecithin, sphingomyelins and galactolipids). For a sixth experiment (rabbit 25) it was further exhaustively extracted with alcohol for removal of cephalin.

The commercial product was kept in chloroform as a stock solution. The purified preparations were kept in ether as stock solutions, from which they were

8. MacLean, H.: J. Path. & Bact. **18**:490, 1914.

precipitated with acetone just before use in order to eliminate the possibility of inclusion of fatty acids split by storage (Boyd[9]). The stock solutions were stored in the ice chest. The materials for injection were measured fresh each time, freed from solvent by evaporation over a water bath, made into 5 per cent emulsions in 5 per cent dextrose and sterilized for two hours in a steam sterilizer. Adult nonpregnant female rabbits were used, and the injections were made in the aural vein. They were given at the rate of one every twenty-four hours, six days a week. The doses were increased gradually from 0.01 Gm. to constant levels varying from 0.4 to 1.0 Gm. daily (i. e., from 8 to 20 cc.). The injections were given slowly over a period of several minutes, and under those conditions, as Sjövall[6] found, no untoward effects were noted. The treatment by daily injections was continued over intervals of time which varied from five to seventy-four days. The animals were kept separately in large cages and were given a liberal diet of oats, carrots and a variety of greens, with water ad libitum. Normal controls were kept similarly. All were weighed once a week. All were clipped when the environmental temperature became sufficiently high to cause elevation of the white blood cell count in a control known to be susceptible. Pearce and Casey[10] demonstrated the seasonal effects which may occur in the blood cell counts of rabbits.

Total and differential white cell counts were made several times a week in all of the experiments. The differential counts were made on 200 cells by the supravital technic. The red cell counts were made infrequently at first. Since they revealed unexpected modifications, two experiments (rabbits 35 and 36) with lecithin from which contaminants known to influence hemolysis had been extracted were added to the series. In these two experiments detailed studies of the red cells were made at the time of each white cell count. Because of the great lability of rabbit's blood incident to circulatory fluctuations, all total counts were made in duplicate, with a third pipet in reserve for instances of disagreement. Trenner automatic pipets and Levy-Hausser counting chambers were used throughout. Except when the blood was followed over a twenty-four hour period after a single injection, the sample for study was always drawn a few hours before an injection.

In the two experiments with detailed study of the red cells the following procedures were carried out: total red cell counts (Hayem's solution), platelet counts (direct counts from the same counting chamber specimens used for the red cell counts), reticulocyte counts (wet counts on 1,000 red cells from pipet contents diluted with 1 per cent brilliant cresyl blue in saline solution and shaken automatically), hemoglobin determinations (Klett hemoglobinometer, calibrated against a standard which establishes 100 per cent as equivalent to 17 Gm. of hemoglobin in 100 cc. of blood), duplicate hematocrit determinations (Van Allen hematocrit tubes centrifuged for forty-five minutes at 2,700 revolutions per minute) and measurements of cell diameters. The diameters were measured on 50 cells in supravital smears as they passed across the field of an ocular micrometer. While this represents an unsatisfactorily small number of cells per count, it is believed that the frequency of the counts, together with the grouping of them into larger weekly units (table 2), results in information as valuable as that which could be obtained from counts of larger numbers of cells made at less frequent intervals.

The fragility of the red cells to water was measured from time to time in these two experiments by removing drops of blood from an ear by fine glass rods

9. Boyd, E. M.: J. Biol. Chem. **121**:485, 1937.
10. Pearce, L., and Casey, A. E.: J. Exper. Med. **52**:39, 1930.

and immediately submerging them in small tubes containing standard dilutions of saline solution. This method was chosen in order to avoid the stimulus to erythropoiesis which might result from frequent removal of amounts of blood large enough for fragility tests made in the usual manner. The control tests agreed within normal limits, not only at the time of a single determination but from day to day.

The icteric index was determined in these two experiments on 1 to 2 cc. of blood withdrawn from an aural vein before an injection. The determinations were

TABLE 2.—*Weekly Averages of the Data Concerning the Erythrocytes of the Two Rabbits (35 and 36) That Were Given Daily Injections of Lecithin from Which Contaminating Hemolysins Had Been Extracted*

Interval of Time	Average Daily Dose of Lecithin, Gm.	Total Count: Red Blood Cells	Total Count: Reticulocytes	Hemoglobin Content, Percentage	Hematocrit Reading, Percentage	Average Cell Volume, Cubic Microns	Average Cell Diameter, Microns	Indexes *: Color	Indexes *: Volume	Average Cell Thickness, Microns †
				Rabbit 35						
May 5-16	Control	5,778,333	108,480	59	40.7	70.5	8.8	1.0	1.00	1.19
to 24	0.16	5,423,333	83,283	60	41.6	76.7	8.0	1.1	1.07	1.55
to 30	0.27	5,247,500	139,830	57	35.5	67.6	8.2	1.1	0.95	1.32
to June 5	0.30	5,586,500	147,889	57	37.5	67.2	8.9	1.0	0.94	1.10
to 13	0.39	4,530,000	217,796	55	30.3	66.9	8.4	1.2	0.94	1.25
to 19	0.46	4,462,500	534,805	51	28.8	64.5	9.0	1.1	0.90	1.05
to 27	0.50	4,083,333	437,082	47	25.7	62.9	8.5	1.1	0.88	1.11
to July 4	0.50	4,362,500	390,575	47	28.2	64.6	8.5	1.1	0.91	1.18
to 11	0.50	4,147,500	257,223	48	27.8	66.9	...	1.1	0.94	....
to 17	0.50	4,232,500	449,393	45	26.5	62.4	9.1	1.0	0.88	1.00
to 21	0.50	4,737,500	365,873	50	28.5	60.2	8.0	1.0	0.84	1.25
				Rabbit 36						
May 3-16	Control	5,478,333	59,563	59	33.6	61.3	8.0	1.0	1.00	1.22
to 20	0.15	5,290,000	52,900	58	33.1	62.6	7.3	1.0	1.00	1.50
to 26	0.29	5,002,500	105,777	56	....	....	7.3	1.0	....	....
to 31	0.39	4,405,000	50,410	55	28.4	64.5	7.0	1.2	1.03	1.70
to June 6	0.40	4,610,000	191,270	51	26.5	57.5	7.8	1.0	0.92	1.20
to 14	0.40	4,256,666	224,058	49	25.8	60.7	8.5	1.1	0.97	1.10
to 20	0.40	4,275,000	283,375	50	27.0	63.2	8.5	1.1	1.01	1.15
to 28	0.40	4,071,666	358,260	47	25.1	61.6	8.2	1.1	0.99	1.20
to July 3	0.40	4,230,000	155,520	44	26.8	63.3	8.5	1.0	1.01	1.12
to 10	0.40	4,476,666	196,258	47	27.5	61.4	8.4	1.0	0.98	1.13

* The indexes are based on the control values for each rabbit as normal for that rabbit.

† The thickness was estimated fom the chart devised by von Boras for the calculation of the thickness of human erythrocytes, as reproduced by R. L. Haden (Principles of Hematology, Lea & Febiger, Philadelphia, 1940).

made only at infrequent intervals in order to avoid the effects of too frequent withdrawal of blood. The serum was treated with two volumes of absolute alcohol, centrifuged and the supernatant fluid matched against dichromate standards. The urine (either the first voiding after an injection, or the twenty-four hour voidings) was also frequently tested in these two experiments for specific gravity, sugar (Benedict's qualitative solution), albumin (nitric acid and heat), blood (guaiac test) and bile pigments (Gmelin's and Rosenbach's methods).

All of the animals were killed eighteen to twenty-four hours after the last injection by intravenous administration of soluble pentobarbital. The inferior vena cava was cut at once and the blood allowed to drain with the animal held upright.

The spleen and the liver were then weighed (table 1), and all organs were examined for gross lesions. The tissues were saved routinely in Bouin's fixative. Blocks of the erythropoietic organs were also saved in Kingsley's [11] and Müller's fixatives, and scrapings were studied supravitally. Staining for fat was usually done on sections from the blocks fixed in Bouin's solution. All sections were stained routinely with hematoxylin and eosin; Kingsley's stain [12] was employed, in addition for the erythropoietic tissues, including the sections of marrow used for counts.[13] Histochemical tests for iron (Key's technic for the prussian blue reaction) were carried out both before and after unmasking. Unmasking was accomplished both with 3 per cent nitric acid in alcohol and with trypsin employed at different intervals of time according to the section.

EXPERIMENTAL DATA

Daily doses of as much as 0.8 Gm. of either the commercial or the purified lecithin were well tolerated over long periods. The animals retained their weight or gained and seemed active and normal in every respect. Greater doses were given only in the case of the commercial lecithin without further purification. One animal (24) that had tolerated smaller doses for weeks became asthenic and lost appetite after three or four doses of 1.0 Gm. One (23) that was not known to be pregnant was found moribund eighteen hours after a single injection of 1.0 Gm., and autopsy revealed dead fetuses. Both of these animals had marked central fatty necrosis of the liver, in contrast to the absence of demonstrable fat in the livers of the other animals of the series.

White Blood Cells.—It is planned to present the data obtained from serial counts following single injections in these experiments in greater detail elsewhere in order that they may serve as control counts for a different series of studies. It is sufficient for the purposes of this report to state that immediately after an injection, whether in an animal previously given injections or not, a transient and slight increase of the total white cell count occurred, involving both neutrophils and lymphocytes, but accompanied by definite monocytopenia. Within about one and a half hours after the injection, the lymphocytes also decreased slightly. Usually the counts gradually returned to the preinjection levels within twelve hours.

While the immediate effects of the injections were circulatory in nature and transient, the daily curves usually showed gradual changes, irrespective of the purity of the material injected. These consisted of moderate increases in the total white cell counts, dependent largely on slight increases in lymphocytes and increases of variable degrees in monocytes. The elevation of monocytes became evident between the third and fourteenth days of the injections, i. e., with daily doses of lecithin which varied from 0.2 to 0.5 Gm. The elevation of lymphocytes was slightly more sluggish in making an appearance. These changes in the white cell counts are quite in agreement with those found previously in a series of control counts made on rabbits treated with lecithin before inoculation with tubercle bacilli.[14] When these studies are coupled with those studies, it seems definite that intravenous injections of egg lecithin stimulate the circulating mononuclears, usually affecting both lymphocytes and monocytes, but the latter more consistently than

11. Kingsley, D. M.: Folia haemat. **57**:87, 1937.

12. Kingsley, D. M.: Stain Technol. **10**:127, 1935.

13. Epstein, R. D., and Tompkins, E. H.: A Comparison of Techniques for the Differential Counting of Bone Marrow (of the Guinea Pig), Am. J. Med. Sc., to be published.

14. Tompkins, E. H.: Am. Rev. Tuberc. **33**:625, 1936.

the former. Figures 1 and 2 present the blood curves of the 2 rabbits (35 and 36) that received daily injections of lecithin extracted for water-soluble, acetone-soluble and ether-insoluble impurities. Platelet counts were also made for these 2 animals but are not charted. They increased slightly by the end of the second week and remained elevated thereafter.

Red Blood Cells.—By the third week of the injections the red cell counts were found to have decreased about 1,000,000 cells in each experiment, and there was a corresponding decrease in hemoglobin, with nucleated red cells appearing intermittently. The daily dose of lecithin by that time varied from 0.2 to 0.6 Gm. The counts and hemoglobin levels remained fairly stationary thereafter.

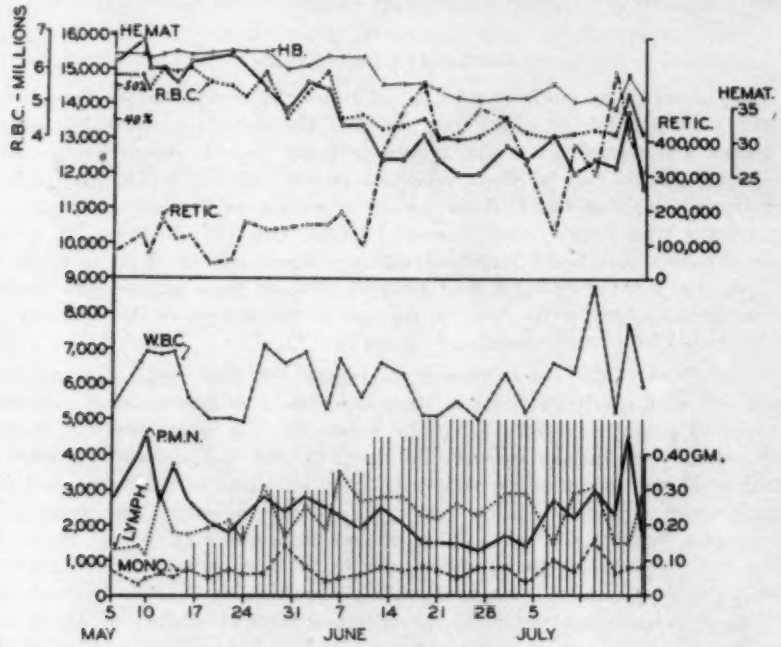

Fig. 1 (rabbit 35).—Curves of the total, reticulocyte and differential (absolute numbers) blood counts and of the hemoglobin and hematocrit readings. The different curves are correlated with the daily injections of lecithin.

In this figure and in figure 2 the white blood cell curves and the daily doses of lecithin are shown below the horizontal line (. - - - . white cell counts; . ——— . neutrophils; o . . . . . o lymphocytes; x - - - x monocytes). The scale at the left represents thousands per cubic millimeter.

Above the horizontal line, the red cell curves are shown (■ . . . . . ■ red cell counts, with scale at the upper far left—millions per cubic millimeter; o . - . - . o reticulocyte counts, with scale at the right—hundred thousand per cubic millimeter; x ——— x hemoglobin readings, with scale at the upper inner left—grams per hundred cubic centimeters; □ ——— □ hematocrit readings, with scale at the far right—volumes per cent).

It was because of these unexpected findings that the two experiments (rabbits 35 and 36) were carried out with lecithin after extraction of fatty acids, lysolecithin and cholesterol, and with more detailed studies of the red cells. Table 2

and figures 1 and 2 present most of the data of these two experiments. On the fourteenth and thirteenth days of the injections, respectively, i. e., with daily doses of 0.3 and 0.4 Gm. of lecithin, the red cell counts and the hematocrit and hemoglobin readings began to decrease slowly. Reticulocytosis developed by the end of the third week of the injections (when the daily doses were 0.45 and 0.40 Gm., respectively) and increased precipitantly to remain at elevated levels throughout the remainder of the experiment. The red cell counts and the hematocrit and hemoglobin values then became stationary at the levels they had attained before this occurred. Nucleated red cells appeared in the circulation in small numbers. The erythrocytes themselves appeared unusually flat and malleable in the supravital smears, with a

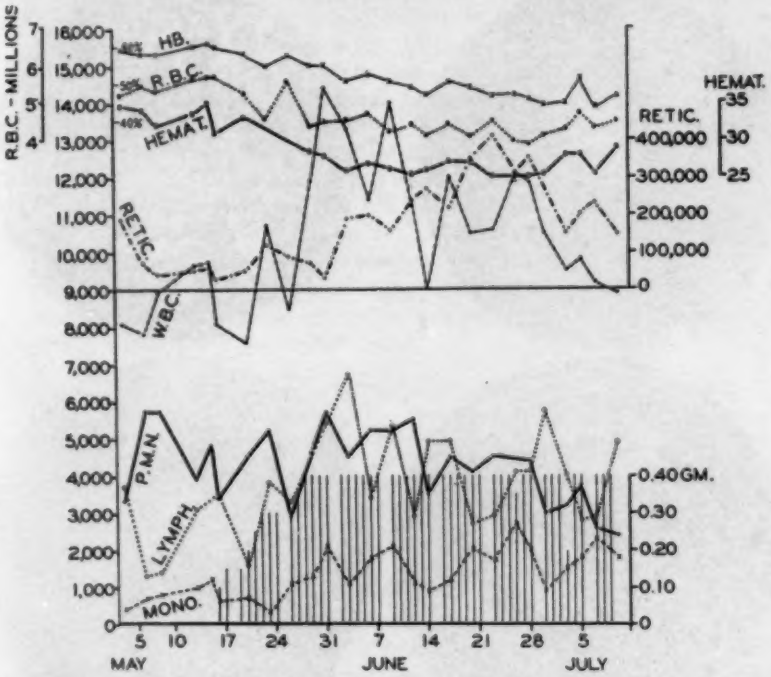

Fig. 2 (rabbit 36).—Curves of the total, reticulocyte and differential (absolute numbers) blood counts and of the hemoglobin and hematocrit readings. The different curves are correlated with the daily injections of lecithin.

tendency toward leaflike and sickle-shaped forms (figs. 3 *A*, *B* and *C*). Such forms often appeared in clusters in the supravital smears. They did not increase in number, however, when the smears were left in the hot box overnight. Variability in size became evident after the onset of reticulocytosis. The distribution curves of the diameters of erythrocytes (Price-Jones) were at first shifted slightly toward the left only but later toward both the left and the right. The average diameters, based on all of the measurements for each week of the experiment, also reflect these changes. The volume indexes increased at first and later decreased. The color indexes remained at unity or above. The weekly averages of these various data are recorded in table 2.

Although the fragility readings did not deviate beyond normal limits, they were consistently higher than those obtained before the injections were started, and the

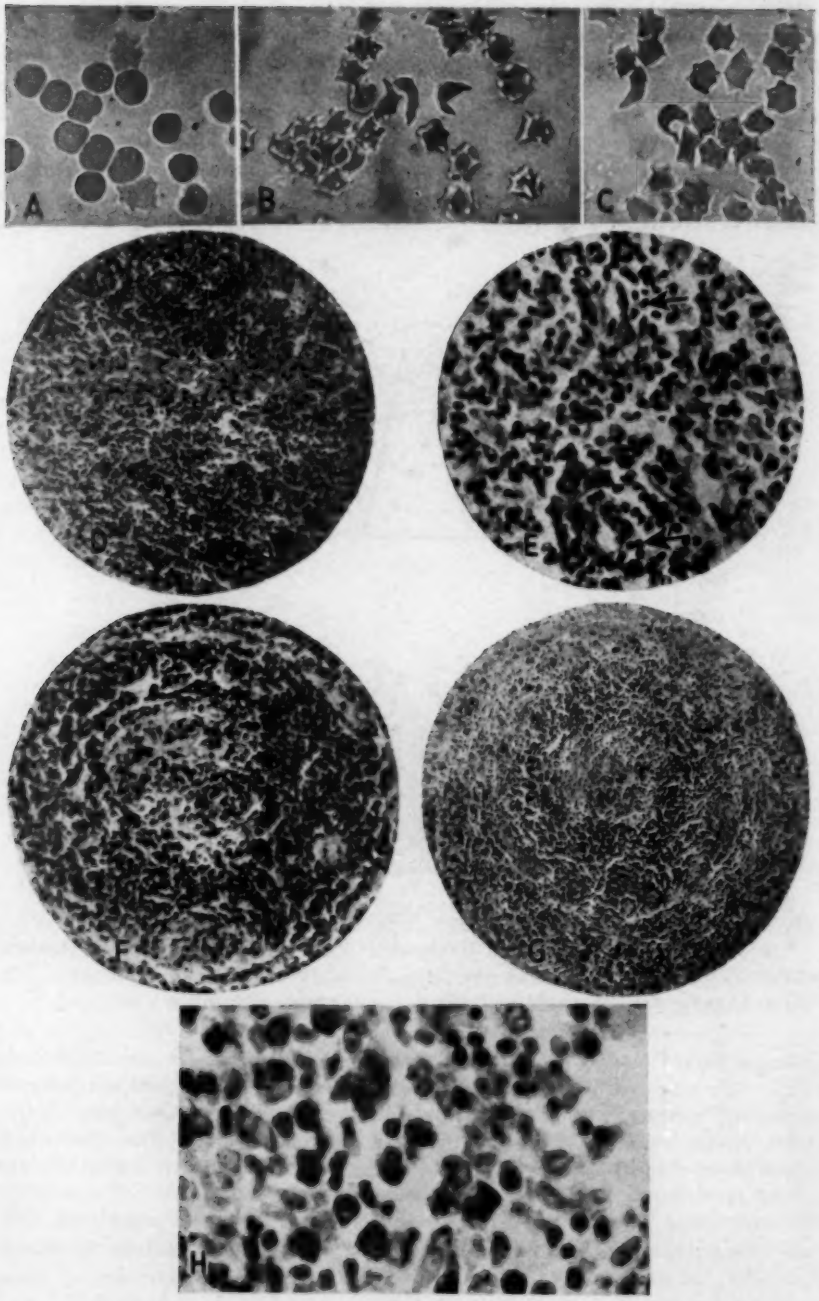

Figure 3

(See legend on opposite page)

differences between the points of beginning and complete hemolysis were slightly greater. The icteric indexes increased slightly, 6 units being the maximum reading at any time. The urine was consistently negative to the guaiac test, was rarely faintly positive to the Rosenbach test for bile pigment and contained traces of sugar and albumin only during the last two weeks of the experiment, when the environmental temperatures were exceptionally elevated.

Pathologic Changes.—The general nutrition was excellent, with abundant subcutaneous and intra-abdominal fat. The fur was thick and silky, and the corneas were normal in appearance.

The major findings were in the hemopoietic organs and were relatively proportional to the maximum daily dose of lecithin (table 1). Daily amounts greater than 0.4 Gm. were apparently necessary for gross changes, although hematologic and morphologic changes occurred with that dose. The femoral marrow was dark red and voluminous but not appreciably firmer than normal. The lymph nodes appeared normal grossly. The spleen was dark purple, firm, uneverted when cut and noticeably smooth in texture with inconspicuous markings, and weighed about three times the average for the normal spleen under the same conditions. The liver weighed more than normal and was somewhat darker than normal in color, except in the rabbits which received the maximum doses of crude commercial lecithin (1.0 Gm. daily). In those it was pale and fatty.

The sections of the hemopoietic organs presented alterations of considerable interest, especially those of the spleen. The latter will therefore be described in detail and the others only as related to them.

EXPLANATION OF FIGURE 3

A (rabbit 35), supravital blood smear (two hours old) on the fifty-seventh day of the experimental injections. The erythrocytes appeared abnormally malleable with a marked tendency toward leaflike and sickle-shaped forms. Two leaflike forms are illustrated. × 718.

B and *C* (rabbit 36), supravital blood smears (two hours old) on the fifty-seventh day of the experimental injections. Elongated and sickle-shaped erythrocytes of the type illustrated frequently occurred in clusters. × 718.

D (rabbit 35), spleen. The splenic pulp was crowded with macrophages and exhibited extramedullary hemopoiesis. The lymphoid nodules were markedly reduced in size and numbers, and the sinusoids were greatly compressed. × 112.

E, higher magnification of *D*. The marked infiltration of macrophages and the depletion of lymphoid tissue are evident. The arrows point to arteries about which are the remnants of lymphoid nodules. × 322.

F (rabbit 35), spleen (different area from that shown in *D* and *E*.). A malpighian corpuscle is seen. A large mass of homogeneous acidophilic material is shown within the concentric sinusoids of the corpuscle (lower part of the figure). Smaller masses are scattered elsewhere around the periphery of the nodule. × 170.

G (rabbit 25), spleen. A malpighian corpuscle is shown. Characteristic masses of homogeneous acidophilic material are shown within the concentric sinusoids of the corpuscle. × 73.

H (rabbit 35), spleen. Same as *D* and *E*. Extramedullary hemopoiesis occurred diffusely. A cord densely infiltrated with macrophages and with myeloid cells is shown bordering a sinusoid in which there is active erythropoiesis. × 718.

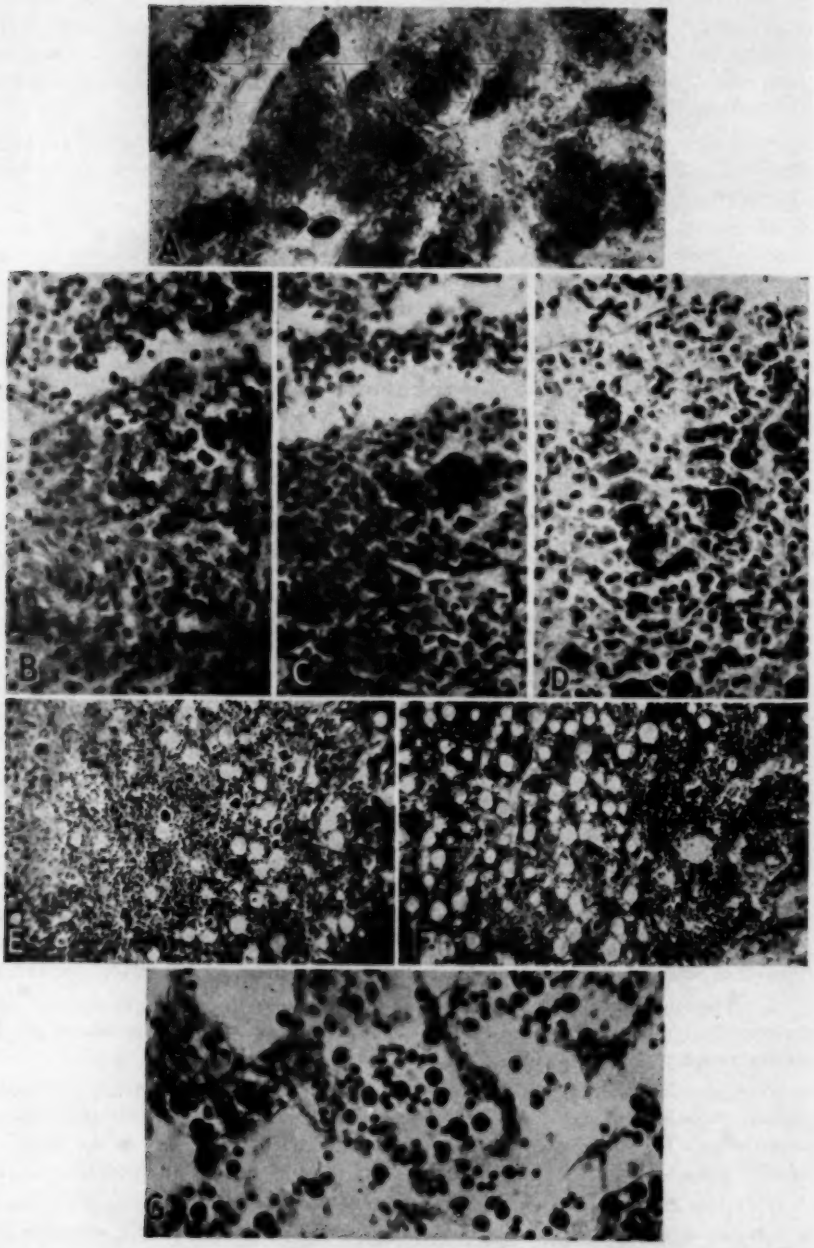

Figure 4

(See legend on opposite page)

The splenic tissue was densely packed with cells, with a consequent inconspicuousness of the sinusoids, both because they themselves shared in the infiltrations and because they were compressed by the markedly infiltrated and thickened cords between them (fig. 3*D*). The compactness of the infiltrations, coupled with a reduction of the malpighian corpuscles to the point of obliteration in some cases, lent a hemogeneity to the picture that was often difficult to recognize as splenic (fig. 3*E*).

Where the corpuscles were not yet reduced practically to arterioles and scattered lymphocytes, they were frequently surrounded by broad bands of homogeneous acid-staining material (figs. 3*F* and *G*). This material was situated within the concentric peripheral sinusoids and was similar to that which Rich [15] noted in the spleen in cases of sickle cell anemia.

The cells which packed the cords and sinusoids so densely and which replaced the areas originally occupied by lymphoid tissue consisted of a mixture, in which, however, cells of one type invariably occurred in great abundance. These cells,

15. Rich, A. R.: Bull. Johns Hopkins Hosp. **43**:398, 1928.

Explanation of Figure 4

A (rabbit 25), liver stained with sudan IV and sudan III. The parenchymal cells contained little fat. The Kupffer cells were increased in number and varied in contents; many were swollen with large deposits which stained an apricot color (two such are illustrated); none were found with the deep color characteristic of neutral fats. × 718.

B (rabbit 35), spleen stained by Key's technic for the prussian blue reaction to iron. The sinusoids (upper part of the illustration) were filled with material which stained dark blue. The macrophages of the pulp, on the other hand, rarely reacted to the histochemical test. At times the deposits in them stained faintly blue, but the greater proportion retained their inherent brownish yellow color. Darkly stained deposits were extremely rare. × 322.

C, same as *B*. The section was treated with 3 per cent nitric acid in alcohol before it was stained for iron. This treatment unmasked large amounts of iron not stainable previously. The contents of the sinusoids stained much the same as in *B*, but the deposits in the macrophages stained blue far more frequently. Many were still brownish yellow, but the majority were tinted with variable degrees of blue, so that they ranged in color from pale green, through the pale and deeper blues, to a few very dark blue ones. × 322.

D, same as *B*. The section was treated with trypsin before being stained for iron. The amount of iron unmasked by this treatment was still greater than that shown in *C*. The sinusoids appeared as in *B* and *C*. The macrophages contained few deposits that were still brownish yellow. Most of the deposits varied in color from medium to dark blue. × 322.

E (rabbit 35), center of the bone marrow from the femur, showing marked hyperplasia. Kingsley's technic; × 73.

F (rabbit 36), center of bone marrow from the femur, showing hyperplasia of a slightly less degree than that illustrated in *E*. Kingsley's technic; × 73.

G (rabbit 35), mesenteric node. The sinusoids were loaded in increasing numbers from cortex to medulla with large basophilic nongranular cells. The cytoplasm of most of these cells was intensely blue, but paler cells were intermingled throughout. Kingsley's technic; × 322.

which were always present regardless of the proportions of the other types and which will be shown to have been present also in all of the hemopoietic tissues, were macrophages. They varied considerably in size. Their cytoplasm always contained an abundance of flocculent deposits, which had an intrinsic brownish yellow hue, and also often contained cellular and nuclear fragments but rarely whole erythrocytes. Binucleated and giant cells were never met. The brownish, flocculent contents of the macrophages proved difficult of analysis. In agreement with the findings of Sjövall [6] in rabbits which had been given lecithin intravenously within twenty-four hours before being put to death they stained an apricot hue with sudan IV and sudan III, and bright red staining was rare. They stained an intense dark blue or dark green with nile blue sulfate but did not stain with osmic acid vapor. Their capacity to stain with nile blue sulfate was unaffected by treatment with various fat solvents (xylene, alcohol, combinations of alcohol, chloroform and ether), and they remained apparently unchanged. This material took acid dyes with difficulty. It stained faintly for iron (fig. 4 *B*) and was therefore presumably hemoglobiniferous in origin, though not in the form of whole erythrocytes and obviously not in the deeply stainable form usually met in splenocytes. In fact, deep staining occurred only in the sinusoids, where innumerable strings and globules, apparently free, were stained dark blue. Prolonged treatment of the sections with nitric acid before staining for iron resulted in considerably deeper staining of the deposits in the macrophages with a variety of hues that ranged in intensity from indigo to pale blue or green (fig. 4 *C*). There remained, however, a variable number of brownish yellow deposits still unmasked. Many of these could be more successfully unmasked by treatment of the sections with trypsin for variable periods before staining for iron (fig. 4 *D*). It is obvious, therefore, that the phagocytosis of whole erythrocytes and the presence of unmasked iron in the spleen were rare except in the sinusoids, but that the macrophages were nevertheless filled with quantities of flocculent material in which masked iron could be demonstrated and which therefore most probably represented fresh fragments of erythrocytes.

In addition to the macrophages which jammed the cords and sinusoids of the spleen, a variety of hemopoietic entities also usually occurred (fig. 3 *H*). These were relatively infrequent in the animal (36) with the smallest daily dose and increased almost proportionately to the dose. Nucleated red cells occurred in great numbers within both sinusoids and cords. They tended to occur in islands and at various stages of development. Myelocytes were frequent in the cords and rare in the sinusoids. They also tended to occur in clusters. Megakaryocytes were also frequent in the cords of the spleen with the greatest degree of hemopoietic activity. Finally, cells of a large type, far more abundant in the nodes (fig. 4 *G*) than in the spleen, were met infrequently in the experiments with the smaller injections but relatively often in those with the larger ones. These large cells were strikingly clear and glassy in supravital preparations and did not stain with neutral red. At times they contained very coarse mitochondria. With Kingsley's stain, their cytoplasm varied from intense basophilia to a clear pale blue, and the nuclei varied correspondingly from stippled to vesicular. Normal reticular cells, as well as the lymphocytes, became relatively decreased as these different forms increased within the splenic tissue. No evidence was obtained of necrosis or fibrosis.

Apart from the livers of the animals given the largest doses of crude lecithin, in which central fatty necrosis occurred, fat was rarely demonstrated in the parenchymal cells. The Kupffer cells (fig. 4 *A*) were often hypertrophied, some-

what increased in number and stained like the macrophages described in the spleen except that they contained relatively less material that stained for iron. Free macrophages and hemopoietic elements as described in the spleen were met infrequently in the hepatic sinusoids.

The nodes, particularly the mesenteric ones, contained many clusters of macrophages similar in all respects to those in the spleen. In addition, the reticular cells often contained small droplets similar to the contents of the macrophages. Erythropoiesis and myelopoiesis were never observed in the nodes. On the other hand, basophilic cells similar to those which were found in moderate numbers in the spleens occurred in overwhelming numbers in the nodes (fig. 4 *G*). They lay in the sinusoids in increasing numbers from cortex to hilus.

The marrow was hyperplastic with considerable reduction of fat spaces as compared with the normal (fig. 4 *E* and *F*). Usually the hyperplasia extended throughout the entire section. Counts made on sections stained by Kingsley's technic revealed a relative increase in erythroid over myeloid elements as indicated by low myeloid-erythroid ratios as compared with the normal (table 1). Macrophages similar to those described in the spleen occurred somewhat in proportion to the size of the daily dose of lecithin.

The lungs appeared normal grossly, but macrophages occurred in greater abundance than normal in the septums and also, rarely, as diffuse accumulations, with constriction of neighboring alveolar spaces. Unlike the macrophages in other sites, these cells when stained supravitally contained the rather small vacuoles which are charactersitic of pulmonary phagocytes normally, and they rarely contained brownish yellow deposits. Periarteriolar accumulations of eosinophils also occurred in varying intensities, irrespective of the purity of the lecithin administered.

The heart appeared large and flabby, with a rather firm left ventricle and a large right one.

The kidneys of the animals which received the crude commercial product contained fine green petechiae or scarring of the cortices. The kidneys of the other rabbits were normal in appearance. The characteristic macrophages occurred sporadically along the tubules or in the glomeruli.

The adrenals appeared smaller than normal, and the sections revealed abnormalities of the zona fasciculata and the zona reticularis which varied considerably in degree. In the extreme degree of change (rabbit 35) the zona fasciculata was abnormally constricted and lacking in fat, while the zona reticularis was abnormally broad because of the hypertrophy and unusually abundant lipoid content of its cells. In this form the changes resembled the findings which Takéda [16] obtained in a proportion of his experiments with guinea pigs. In less involved glands the cells of the zona fasciculata appeared normal and seemed to contain the normal amount of fat, but nests of large cells, with small eccentric nuclei and unusually irregular deposits of neutrai fat, occurred in the inner zone and impinged on the zona reticularis more abundantly than in the adrenals from the controls. Macrophages occurred sporadically.

No consistent changes were found in the ovaries, the uterus or the mammary tissues other than the frequent appearance of clusters of the characteristic macrophages in the adipose tissue surrounding the mammary glands.

16. Takéda, Y.: Compt. rend. Soc. de biol. **116**:1164, 1934.

COMMENT

The findings are obviously different from those of the recognized lipoid storage diseases with their characteristic infiltrations of huge cells filled with material extractable by lipoid solvents and therefore vacuolated and foamy in appearance in fixed tissues. These cells were filled with a substance which reacted to fat stains but which was not extracted in large enough amounts by fat solvents to leave appreciable space in fixed tissues, and which seemed to be linked with hemoglobin-bearing material in such a manner that the iron could be demonstrated only by unmasking with nitric acid or trypsin. These cells were smaller than those in the known lipoid storage diseases. The cells obtained by subcutaneous injections of lecithin were also smaller than those obtained by similar injections of the lipoids concerned in the known storage diseases.[7]

Yet in other respects the experimental pathologic condition bears close resemblances to the lipoid storage diseases. A lipoid was administered in large amounts. The same organs were involved as in the lipoid storage diseases and, to a large extent, in the same manner. The marrow, the lymph nodes, the spleen and the liver contained far greater numbers of macrophages than normal. These phagocytes filled the same spaces in the spleen as in the lipoid storage diseases, i. e., the cords, and there was the same tendency toward depletion of lymphoid tissue. The spleen was greatly enlarged, and there was extramedullary hemopoiesis. Sjövall [6] also observed the invasion of the sinusoids and cords of the spleen by phagocytic cells in the two experiments in which he gave repeated injections of lecithin. He considered these cells to be "lipophages" and commented on the parallelism with the storage phenomena of Niemann-Pick disease. In their content of iron these cells also resemble the features often observed in cases of Gaucher's disease.

From another point of view, however, the findings may be regarded as typical of those of any chronic hemolytic anemia. There was gradual decrease in the number of erythrocytes, the hemoglobin content and the hematocrit reading. These changes were ultimately checked by increased erythropoiesis, as indicated by continued reticulocytosis, the occurrence of nucleated red cells in circulation and the extramedullary activity of the spleen in addition to accelerated erythropoiesis in the marrow. These changes were accompanied by spreading of the distribution curves of diameters of erythrocytes (Price-Jones) to both right and left, by slight decreases in volume index without change in color index, and by slight increases in icteric index. Macrophages filled with hemoglobin-bearing particles occurred in great numbers in all of the hemopoietic tissues.

The appearance of the splenic nodules, the malleability and normal fragility of the erythrocytes and the marked erythrophagocytosis, or at least the phagocytosis of fragments of erythrocytes, are features which bear close resemblances to the findings in early cases of that particular form of hemolytic anemia met in sickle cell anemia (Huck [17]; Sydenstricker [18]; Diggs and Ching [19]; Graham and McCarty [20]). The amount of demonstrable iron in the spleen in this disease may be small as compared with the degree of erythrophagocytosis.[21] Peabody and Broun [22] made the same observation in cases of pernicious anemia, and it was also made in these experiments until methods of unmasking were employed.

It seems, therefore, that one is dealing with reactions in the tissues that may be regarded as an admixture of those of a lipoid storage disease and those of hemolytic anemia, both elicited entirely by the administration of a glycerophosphatide.

If one attempts to analyze the basis of the hemolytic features of the picture several possibilities present themselves. Lecithin stimulates the production of macrophages when injected subcutaneously.[7] It seems from these experiments that it does this also when administered intravenously. The mooted question arises as to whether abnormal destruction of erythrocytes may occur due entirely to overactivity of the reticuloendothelial system. That this is not the chief explanation in this instance seems likely in view of the fact that phagocytosis of whole erythrocytes was obviously rare and that, likewise, it was rarely observed in the earlier experiments with subcutaneous injections of lecithin.[7] Furthermore, the tissues from those experiments have been restudied with the technics for the demonstration of iron that were employed in the present series, and they have yielded positive results so rarely as to lead to the conclusion that such instances represent merely the response to hemorrhage occurring occasionally at the time of the subcutaneous injections.

That direct hemolysis with liberation of free hemoglobin into the blood stream may have occurred incident to the injections and given rise to the hemolytic features of the picture also seems unlikely in view of the lack of positive evidence to that effect. Except in the experiments with crude commercial lecithin from which fatty acids and other possible contaminating hemolysins had not been extracted, there were no observable reactions on the part of the animals, no positive results from tests for blood in the urine and no findings in the tissues suggestive of direct hemolysis and liberation of free hemoglobin.

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17. Huck, J. G.: Bull. Johns Hopkins Hosp. **34**:335, 1923.
18. Sydenstricker, V. P.: South. M. J. **17**:177, 1924.
19. Diggs, L. W., and Ching, R. E.: South. M. J. **27**:839, 1934.
20. Graham, G. S., and McCarty, S. H.: South. M. J. **23**:598, 1930.
21. Diggs and Ching.[19] Graham and McCarty.[20]
22. Peabody, F. W., and Broun, G. O.: Am. J. Path. **1**:169, 1925.

That the lecithin, on the other hand, affected the erythrocytes in such manner that they were more subject to fragmentation and phagocytosis than normal seems likely. The character of the inclusions within the macrophages supports this concept; these inclusions obviously were not whole erythrocytes; yet they had characteristics of those elements. The slight tendency toward sphericity which was found in the circulating erythrocytes supports the same concept. The measurements of diameter were made routinely many hours after the injections, and it is possible that even greater distortion of the cells occurred earlier. Experimental evidence supports the probability that phospholipids in circulation are adsorbed on erythrocytes (Bloor [23]); Ponder [24] showed that erythrocytes become spheroidal in suspensions of lecithin; various investigators (Haden [25]; Dameshek [26]; Castle and Daland [27]; Singer [28]) have demonstrated that sphericity is associated with increased fragility, and it seems probable that the earlier studies concerning the antagonistic effects of lecithin and cholesterol on the "permeability" of erythrocytes to various hemolytic agents are explainable on a similar basis (Brinkman and von Dam [29]; Kimmelstiel [30]; Spranger [31]). While the fragility of the erythrocytes in these experiments did not exceed normal limits, the fact that the resistance was consistently slightly less after the injections started than in the control measurements is probably significant of some degree of increased fragility. This is especially true in view of the great number of relatively resistant reticulocytes in circulation. Dameshek and Schwartz [32] called attention to the fact that measurements of fragility, as well as those of average cell diameter, represent a composite of the measurements of the more resistant young cells and of the less resistant spheroidal ones.

The findings, therefore, support the concept that the erythrocytes were rendered abnormally subject to fragmentation by the injected lecithin. Fragmentation was undoubtedly further aided by the conditions within the spleen. The cords were greatly hypertrophied by the infiltration of macrophages and the hemopoiesis within them. The sinusoids were correspondingly compressed and, furthermore, were con-

23. Bloor, W. R.: Physiol. Rev. **19**:557, 1939.
24. Ponder, E.: J. Exper. Biol. **13**:298, 1936.
25. Haden, R. L.: Am. J. M. Sc. **188**:441, 1934
26. Dameshek, W.: New England J. Med. **221**:1009, 1939.
27. Castle, W. B., and Daland, G. A.: Arch. Int. Med. **60**:949, 1937.
28. Singer, K.: Am. J. M. Sc. **199**:466, 1940.
29. Brinkman, R., and von Dam, E.: Biochem. Ztschr. **108**:61, 1920.
30. Kimmelstiel, P.: Virchows Arch. f. path. Anat. **282**:402, 1931.
31. Spranger, W.: Biochem. Ztschr. **218**:341, 1930.
32. Dameshek, W., and Schwartz, S. O.: Am. J. M. Sc. **196**:769, 1938.

gested with exceptional numbers of cells similar to those in the cords. The erythrocytes must have met considerable impediment within them, and this, combined with the fact that they were already fragile by virtue of the action of the lecithin on them, may be supposed to have subjected them to an unusual degree of trauma.

How long the direct effects of lecithin on erythrocytes may have lasted between injections is entirely subject to surmise as far as these experiments are concerned. Sjövall [6] expressed the opinion that lecithin is so rapidly absorbed that its effects are inevitably transitory. The chemical studies of Pasternak and Page [33] with injections of cephalin and those of Haven and Bale [34] with labeled phosphatide would at first thought suggest the same probability. These observers found that the glycerophosphatides which they used were removed from the circulation within one half to two hours unless given in far greater amounts than in these studies. However, they found these materials to be deposited largely in the spleen and the liver at first, with a gradual shift later from those sites into other areas. These observations therefore lead to the suggestion that the local effects of lecithin on erythrocytes in the present experiments probably continued far longer in those organs than in the general circulation, with the result that the effects in those sites were practically chronic.

From this discussion, therefore, the conclusions seem justified (1) that repeated intravenous injections of lecithin result in tissue changes which are representative at one and the same time of those in lipoid storage diseases and those due to destruction of erythrocytes, (2) that the former are characterized by stimulation of the reticuloendothelial system but are modified by changes which are due to the destruction of erythrocytes, and (3) that the destruction of erythrocytes is due to fragmentation which is facilitated by the direct effect of lecithin on the resistance of erythrocytes together with unusual stress exerted on them in the splenic and other sinusoidal areas.

SUMMARY

Repeated intravenous injections of lecithin in sufficient amounts cause:

1. A generalized infiltration of macrophages, which contain both lipoids and fragments of erythrocytes but which stain for iron only after unmasking.
2. An increase in the white blood cell count, dependent largely on increases in the numbers of lymphocytes and monocytes.

33. Pasternak, L., and Page, I. H.: Biochem. Ztschr. **252**:254, 1932.
34. Haven, F. L., and Bale, W. F.: J. Biol. Chem. **129**:23, 1939.

3. A decrease in the red blood cell count, accompanied by a decrease of the hemoglobin and hematocrit readings, a slight decrease in volume index, a tendency toward decreased resistance, abnormal malleability of the erythrocytes, a slight increase in the icteric index and a normal color index. These changes eventually are checked and held stationary by continued reticulocytosis, accompanied by circulation of nucleated red cells and spacing of the distribution curves of diameters of erythrocytes (Price-Jones) to the right and the left.
4. Hyperplasia of the bone marrow with increased erythropoiesis, decrease of the myeloid-erythroid ratios and infiltrations of the characteristic macrophages.
5. Splenomegaly associated with (*a*) massive infiltrations of the characteristic macrophages, (*b*) hemopoiesis, (*c*) deposition of acidophilic bands about the peripheries of the malpighian corpuscles and depletion of the corpuscles and (*d*) constriction and congestion of the sinusoids.
6. Infiltrations of young basophilic cells in the lymphoid tissues.

The findings are discussed from the standpoint of their mutual relationships both to the lipoid storage diseases and to hemolytic anemias.

The influence of lecithin on the resistance of erythrocytes and the exposure of the erythrocytes to an exceptional degree of trauma in the spleen are offered in explanation of the hemolytic features.

INFLUENCE OF VITAMIN B_6 AND PANTOTHENIC ACID ON GROWTH OF SARCOMA 180

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Studies from this laboratory [1] have shown that vitamins B_1 and B_2, while having a profound effect on the nutritional state of the mouse, as indicated by caloric consumption and somatic growth, and thus an indirect effect on tumor growth, do not remedy deficiency of a factor the lack of which markedly retards tumor growth (sarcoma 180). The latter factor was relegated to the B_6, or filtrate, fraction of the B complex. Morris and Lippincott [2] extended the studies on the B complex to pantothenic acid and spontaneous mammary carcinoma, and confirmed our finding. The present report, which is a study of the influence of vitamin B_6 and pantothenic acid on the growth of sarcoma 180, was begun when these substances became available in pure form.

EXPERIMENTAL PROCEDURE

In experiment 1 synthetic diet 5, previously described,[1] which contains 25 parts vitamin-free casein, 63 parts starch, 4 parts hydrogenated cottonseed oil, 3 parts agar and 4 parts salt mixture, was used.[3] Nicotinic acid when administered was incorporated in 0.05 per cent concentration. Vitamins B_1, B_2 and B_6 were injected parenterally, each in the amount of 0.01 mg. per mouse per day. In experiments 2 and 3 the diet consisted of 25 parts vitamin-free casein, 65 parts starch, 4 parts hydrogenated cottonseed oil, 3 parts agar and 2 parts salt mixture. The vitamin mixture described by Lippincott and Morris was added to this diet in the concentration recommended by the authors.[4] The complete diet used in experiments 2 and 3 differed from that used in experiment 1 essentially in the addition of choline hydrochloride and pantothenic acid. The experimental procedures for measuring

From the Chemical Laboratory, Santa Barbara Cottage Hospital Research Institute.

1. Bischoff, F., and Long, M. L.: Am. J. Cancer **37**:54, 1939.

2. Morris, H. P., and Lippincott, S. W.: J. Nat. Cancer Inst. **2**:47, 1941.

3. The vitamin-free casein used was Borden's Labco vitamin-free casein, and the hydrogenated cottonseed oil was Crisco.

4. Lippincott, S. W., and Morris, H. P.: J. Nat. Cancer Inst. **2**:39, 1941.

caloric consumption, the technic of inoculation and the statistical analysis of the data on the tumors have been previously described.[5] In each experiment, 15 to 16 mice were used per diet group, 60 mice being used in experiment 2 and 48 mice in experiment 3. In experiment 1, a number of mice succumbed on the diets completely deficient in vitamins, and the data on the tumors are calculated for the surviving mice.

RESULTS

The data in the accompanying table give the period during which the mice were maintained on each dietary regimen, the relation of the day of inoculation with tumor to the start of the regimen, the mean net change in somatic weight, the mean diameter of the tumors at the end of the

Influence of Vitamin B_6 Deficiency and Pantothenic Acid Deficiency on the Growth of Sarcoma 180

Experiment	Diet *	Days on Dietary Regimen	Day of Inoculation (on Regimen)	Mean Net Change in Somatic Weight, Gm.	Mean Diameter of Tumors, Mm.	Mean Consumption of Calories	
						Calories	Days
1	Synthetic diet 5	30	10	−0.2	5.9 ± 0.8	187	30
	Synthetic diet 5 + B_6	30	10	−0.6	8.8 ± 0.6	208	30
	Synthetic diet 5 + B_1, B_2, nicotinic acid	30	10	−2.0	7.0 ± 0.9	304	30
	Synthetic diet 5 + B_1, B_2, B_6, nicotinic acid	30	10	−2.3	12.2 ± 0.6	285	30
	Calf meal	30	10	+1.5	12.4 ± 0.7		
2	Calf meal	20	1	−0.3	19.0 ± 0.8	250	20
	Synthetic diet 10	20	1	+0.4	19.0 ± 1.0	242	20
	Synthetic diet 10 — B_6	20	1	−2.0	12.5 ± 0.9	214	20
	Synthetic diet 10 — pantothenic acid	20	1	−1.7	18.0 ± 0.9	228	20
3	Synthetic diet 10	24	9	−1.6	15.7 ± 0.9	247	21
	Synthetic diet 10 — B_6	24	9	−2.4	10.2 ± 1.0	221	21
	Synthetic diet 10 — pantothenic acid	24	9	−2.7	15.2 ± 0.9	246	21

* Note that diet 5 is vitamin free, while diet 10 contains vitamins of the B complex.

dietary regimen and the mean caloric intake for the whole or the last period of the dietary regimen. The mean net change in somatic weight is calculated by subtracting the mean tumor weight from the mean net change in body weight. The statistic is the standard deviation of the mean.

In experiment 1 the addition of vitamin B_6 to the diet either with or without the other vitamins did not significantly influence the caloric intake but did significantly influence the rate of tumor growth. The effect is particularly striking in the series of mice which received vitamins B_1, B_2 and nicotinic acid. The influence of vitamins B_1, B_2 and nicotinic acid in increasing caloric intake and decreasing loss in body

5. Bischoff, F.; Long, M. L., and Maxwell, L. C.: Am. J. Cancer **24**:549, 1935.

weight without affecting tumor growth confirms the results of experiments previously reported.[6]

In experiments 2 and 3 the withdrawal of pantothenic acid from the diet was not followed by a significant reduction in caloric intake or in tumor growth but was followed by a slight reduction in body weight. Under the same conditions of experimentation the withdrawal of vitamin B_6 reduced tumor growth markedly.

In experiment 3 the mean weight of the thymus gland for 9 control mice was 33.0 ± 3 mg.; that for 10 mice on the diet deficient in vitamin B_6 was 22.6 ± 2.2 mg.

In experiment 1 all mice were returned to an adequate diet at the end of the thirty day period on the dietary regimen; in all cases the rate of growth of the tumors returned to the rate of the controls within a five day period; i. e., the mean diameter of the tumors of each group increased 4.1 to 5.0 mm. in five days. This procedure shows that the inherent growth characteristics of the tumor were not affected. In experiment 2 the tumors were weighed at the end of the period of the dietary regimen to ascertain whether the diameter and weight of the tumors were correlated to the degree ascertained for control tumors. In experiment 3 the tumors were reserved for histologic examination. The sections were given to the histologist (J. J. R.) in code. They were examined and classified as to: (1) tendencies toward invasion of fat or muscle; (2) tendencies toward invasion of fat; (3) necrosis; (4) site of necrosis, peripheral or central; (5) mitosis; (6) vascularity; (7) presence of odd mononuclear cells. Items 1, 2, 3, 5 and 6 were graded as to degree and the standard deviation of the mean was calculated for the mean result of each group. Items 4 and 7 were analyzed statistically by the all or none effect. The only significant finding was that the tendency to invade fat was significantly less for the tumors of the mice on the deficient diet. Since the tumors were growing at a greatly reduced rate, this finding would probably be without significance. Of interest is the negative finding for differences in degree of necrosis, mitosis and vascularity.

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6. Jones[7] has reported for the Jensen rat sarcoma that deprivation of vitamin B_1 apart from caloric restriction affected the future course of the tumor. This deduction is based on the regression of tumors of rats placed on an adequate diet after a period of B_1 deficiency with attending caloric restriction. In these experiments the controls were not placed on a diet restricted to the caloric intake of the rats which were on the diet deficient in vitamin B_1 and the deduction is therefore in our opinion unwarranted, as one of us, in collaboration with Long and Maxwell,[5] has previously reported that the characteristics of sarcoma 180 may be affected by marked caloric restriction on a diet otherwise adequate in specific nutrients. Bischoff and Long.[1]

7. Jones, J. L.: Cancer Research **2**:697, 1942.

COMMENT

The question always arises as to the significance of retardation in tumor growth (tumor diameter or weight). In the present experiments the addition of vitamin B_6 to a diet containing the other listed members of the B complex produced tumors which exceeded those of the groups deficient in vitamin B_6 by 200 to 250 per cent of weight.

In our experiments the longest period on the dietary regimen was thirty days. In working with a spontaneous tumor Morris and Lippincott were able to extend this period, but the difference in results obtained for these tumors through pantothenic acid is not entirely explained on this basis. The negative results in our experiments with pantothenic acid may be due to failure to deplete endogenous stores of pantothenic acid. The results for vitamin B_6 are so clearcut that they establish the essentiality of this substance for the maximum growth of sarcoma 180.

SUMMARY

In three series of experiments comprising 122 Marsh-Buffalo mice, the maintenance on a synthetic diet containing vitamins of the B group other than B_6 produced a marked and significant decrease in the rate of tumor growth, which was corrected by the addition of vitamin B_6 without the caloric intake being significantly changed.

In two series of experiments running concurrently with the experiments with vitamin B_6 (31 additional mice), pantothenic acid deficiency was without influence on the rate of tumor growth.

In a single experiment comprising 30 mice, the addition of vitamin B_6 to a diet otherwise completely deficient in the B complex produced a significant increase in tumor growth.

ANOMALIES OF THE AORTIC ARCH

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Anatomic variations of the aortic arch in man pursue definite patterns. It is the purpose of this presentation to consider the development of the more common anomalies and to present illustrative cases of each type. The cases described are taken from the last 5,800 necropsies performed at the Jefferson Medical College Hospital from 1912 to 1942, inclusively.

EMBRYOLOGY

The development of the normal aortic arch and its main branches has been described in detail by Congdon [1] and needs to be considered only briefly here. Of the six arches which develop on each side of the notochord, the first and second do not contribute to the aortic arch or to its immediate branches. The origin of each third arch becomes the common carotid artery. The proximal portion of the right fourth arch is incorporated in the right subclavian artery, while the entire left fourth arch remains as the definitive aortic arch. The fifth arches form no permanent vessels in the adult. The proximal portions of the sixth arches become the pulmonary arteries while the distal portion on the right side disappears and that on the left becomes the ductus arteriosus.

ANOMALIES

Not always do the arches follow the specific normal courses just outlined. When, however, the complicated development and the delicate mechanisms which govern the evolution are considered, it is indeed surprising that deviations from the normal pattern are not more frequent. The major anomalies may be divided into five definite groups. In approximately the order of their frequency, the types are:

1. Coarctation of the aorta. This is perhaps the most familiar anomaly. It is a constriction of the aorta, either partial or complete, at, above or below the point at which the ductus arteriosus or the ligamentum arteriosum enters the aorta (fig. 1, *I*).

2. Patent ductus arteriosus. In this anomaly the ductus arteriosus, instead of obliterating as it should shortly after birth, remains patent throughout adult life (fig. 1, *II*).

From the clinical laboratories, Jefferson Medical College Hospital.

1. Congdon, E. D.: Contrib. Embryol. **68**:47, 1922.

3. Posterior right subclavian artery. Normally, the dorsal portion of the right fourth aortic arch, between the entrance of the seventh cervical intersegmental artery and the descending aorta, obliterates, and the anterior portion becomes the definitive right subclavian artery. Sometimes the anterior portion obliterates (fig. 1, *III*). The definitive right subclavian artery then originates from the last portion of the aortic arch, making its exit immediately distal to the left subclavian

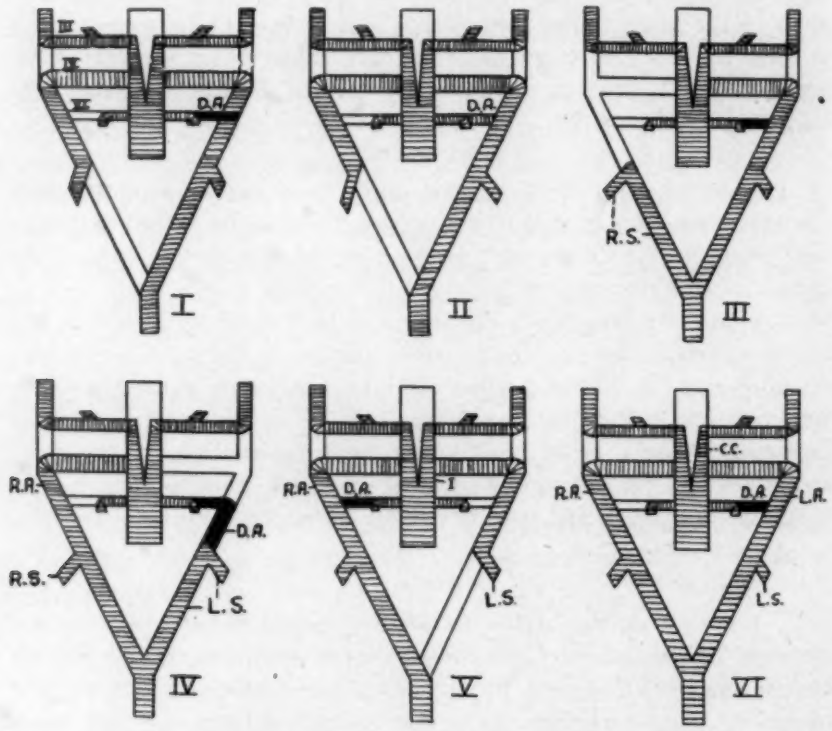

Fig. 1.—Diagrammatic sketch of the third, fourth and sixth arches. Shaded areas represent the definitive arteries; unshaded areas, the primitive portions that disappear. *I* represents coarctation of the aorta opposite an obliterated ductus arteriosus, *D.A. II*, shows a patent ductus arteriosus, *D.A. III.*, shows a posterior right subclavian artery, *R.S. IV*, represents a right-sided aortic arch, *R.A.*, with a posterior left subclavian artery, *L.S. D.A.* is the obliterated ductus arteriosus; *R.S.*, the right subclavian artery. *V*, portrays a right-sided aortic arch, *R.A.*, with a left innominate artery, *I*, and an anterior left subclavian artery, *L.S.* The obliterated ductus arteriosus, *D.A.*, is on the right side. *VI*, shows a double aortic arch, *R.A.* (right aortic arch) and *L.A.* (left aortic arch). *D.A.* is the obliterated ductus arteriosus on the left side, *C.C.*, the common carotid arteries and *L.S.*, the left subclavian artery with a right subclavian artery symmetrically opposite.

artery. It pursues its course to the right side between the esophagus anteriorly and the vertebral bodies posteriorly.

4. Right-sided aortic arch. In this anomaly the right fourth aortic arch becomes the definitive aortic arch while the left one partially disappears. Most frequently that portion of the left arch between the aortic sac and the left seventh cervical intersegmental artery disappears (fig. 1, *IV*). The left subclavian artery then arises as the last branch of the right arch. Less frequently, the posterior portion of the left aortic arch between the seventh cervical intersegmental artery and the descending aorta disappears (fig. 1, *V*). In such instances the left subclavian arises from a left innominate artery.

5. Double aortic arch. This is the least common of the anomalies. Both the right and the left fourth aortic arch persist throughout life, forming a complete arterial collar about the trachea and esophagus (fig. 1, *VI*). There is no innominate artery. Both the right and the left common carotid and subclavian arteries arise independently and symmetrically from their respective arches.

CLINICAL CONSIDERATION WITH PRESENTATION OF CASES

Coarctation.—Although coarctation of the aorta is the most common anomaly, it is not of frequent occurrence. At this hospital there are recorded only 3 cases, giving a frequency of 0.05 per cent.

The symptoms of coarctation may be few and varied, or symptoms may be entirely absent.[2] When present, they are often those of hypertension,[3] which include precordial pain, headache, dizziness, epistaxis and vomiting, or those of cardiac failure. Physical examination reveals enlargement of the heart to the left. Systolic or diastolic murmurs, heard best at the base of the heart and transmitted to the neck or to the intercapsular area, may be present. There is usually hypertension in the upper extremities and marked hypotension in the lower ones. When a collateral circulation is well established, the vessels originating proximal to the constriction may be felt and seen to pulsate. The diagnosis is confirmed by roentgenographic examination, showing erosions of the inferior margins and posterior portions of the ribs, or, when these do not appear, by intravenous injection of diodrast.[4]

The prognosis depends on the degree of stenosis, the extent to which a collateral circulation has been developed and the degree of hypertrophy

2. Abbot, M. E.: Am. Heart J. **3**:392, 1928. Evans, W.: Quart. J. Med. **26**:1, 1933.

3. Fray, W. B.: Am. J. Roentgenol. **24**:349, 1930. Railsback, O. C., and Dock, W.: Radiology **12**:58, 1929.

4. Blumenthal, S., and Davis, D. B.: Am. J. Dis. Child. **62**:1224, 1941. Grishman, A.; Steinbergh, M. F., and Sussman, M. L.: Am. Heart J. **21**:365, 1941.

of the left ventricle. Pregnancy is contraindicated.[5] The causes of death are myocardial failure, rupture of the ascending aorta, endocarditis and cerebral hemorrhage.[6]

CASE 1.[7]—A white man 26 years of age was admitted in shock. Four days previously, while carrying a timber at work, he experienced severe pain in the pit of the stomach. This necessitated rest in bed for two days. On returning to work, he experienced the same pain, but this time it was so severe that he collapsed. Prior to these episodes he was always in perfect health. Physical examination revealed pulsating vessels in the neck, the heart slightly enlarged transversely, a systolic murmur at the apex and blood pressure in the right arm averaging 130 systolic and 60 diastolic.

Necropsy showed constriction of the aortic arch directly opposite the ligamentum arteriosum. It measured 0.8 cm. in outside diameter, while the arch immediately above measured 1.3 cm. and the descending aorta immediately below 1.7 cm. The ascending aorta was 3.5 cm. in diameter at the base and 2 cm. at the level of the innominate artery. The main branches of the aortic arch and the internal mammary arteries were greatly dilated. The diameter of the innominate artery was 1.9 cm., that of the left common carotid artery 0.9 cm. and that of the left subclavian 1.3 cm. The ascending aorta contained at its base and posterior surface a ragged rupture, measuring 2.5 cm. in diameter. This was the starting point of a dissecting aneurysm which extended superiorly to the great vessels of the neck and inferiorly into the pericardial cavity. The latter contained 700 cc. of clotted and fluid blood. The coarctation was increased by the presence of a fibrous diaphragm in the intima opposite the external narrowing. Both ventricles were somewhat hypertrophied, the left measuring 2.5 cm. in thickness and the right 0.75 cm.

CASE 2.—A white woman aged 47 was admitted because of intermittent uterine bleeding of four months' duration. She had no complaints referable to the heart. Physical examination revealed a left-sided cardiac enlargement, a systolic murmur over the pulmonic area and at the base of the heart, and pulsating vessels in the neck. Fluoroscopic examination of the chest revealed no abnormality. A roentgenogram showed cardiac enlargement, a tortuous and dilated aorta and an aortic arch raised to the level of the suprasternal notch. The blood pressure varied from 120 to 150 systolic and from 60 to 100 diastolic. Hysterectomy was performed for carcinoma of the fundus of the uterus, after which the patient died of peritonitis.

Necropsy showed constriction of the aortic arch opposite the ligamentum arteriosum. The circumference of the aorta at this point was 5 cm., while that above was 9 cm. and that below 6 cm. The intima opposite the constriction disclosed a small ridge and dimple. The innominate, left common carotid and left subclavian arteries were definitely dilated. The heart showed relative left ventricular hypertrophy and some dilatation.

CASE 3.—A white boy 2 months of age was admitted with a history of cough for three days and fever for one day. He had been treated for thymic enlargement when 12 days old. Physical examination revealed rapid respiration, indrawing of

5. Mendelson, C. L.: Am. J. Obst. & Gynec. **39**:1014, 1941.

6. Baker, T. W., and Sheldon, W. D.: Am. J. M. Sc. **191**:626, 1936. Blackford, M. L.: Arch. Int. Med. **41**:702, 1928.

7. This case has been previously reported by Stewart, H. L., and Bellet, S.: Am. Heart J. **9**:533, 1934.

the suprasternal notch, marked venous pulsation in the neck, rales in the lungs and cardiac enlargement. A roentgenogram of the chest showed bronchopneumonia and a narrow upper mediastinal shadow.

Necropsy disclosed a heart weighing 74 Gm. The left ventricular wall measured 1 cm. in thickness. The foramen ovale was almost closed. The first part of the aorta and the innominate artery were dilated. The arch opposite the entrance of the ductus arteriosus was moderately constricted, measuring 1.2 cm. in circumference. The aorta above the constriction measured 2.5 cm. in circumference, while below the constriction it measured 2.0 cm. The lumen opposite the narrowing was patent. The ductus arteriosus is not mentioned in the protocol.

Patent Ductus Arteriosus.—Although much less frequently reported than coarctation of the aorta, this anomaly has recently gained much prominence because it is amenable to surgical treatment.

Early in life a retardation of growth may be the only presenting symptom; later, symptoms of cardiac failure or of other complications may be manifest.[8] Physical examination reveals an enlarged heart, a thrill over the precordium, a loud continuous murmur opposite the second and third interspaces, accentuation or reduplication of the second pulmonic sound and occasionally signs of aortic insufficiency. A roentgenogram shows a large heart, a prominent pulmonary conus and a congested pulmonary artery.[9] Intravenous roentgenography has so far been less successful in demonstrating a patent ductus.[10]

The ultimate prognosis, as a rule, is poor. By the age of 40 years, 71 per cent of the patients die from subacute bacterial endocarditis, congestive heart failure or rupture of the pulmonary artery.[8b]

The treatment is medical as long as the patient shows no evidence of retardation of physical development, cardiac hypertrophy, cardiac failure[11] or superimposed subacute bacterial endocarditis.[12] When, however, any of the complications named become manifest, excellent results may be obtained by surgical ligation of the patent ductus arteriosus.

Case 4.—A white man 41 years of age was known to have congenital heart disease. He was otherwise healthy until twelve months previous to the illness in question. Then he began to have recurrent attacks of night sweats, general malaise and a low grade fever. Physical examination showed an enlarged heart, a diastolic and presystolic murmur at the apex, a palpable spleen, petechiae at both ankles, moderate anemia and a blood culture positive for Streptococcus viridans.

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8. (*a*) Gross, R. E.: J. A. M. A. **115**:1257, 1940. (*b*) Bullock, L. T.; Jones, J. C., and Dolley, F. S.: J. Pediat. **15**:786, 1939.

9. Eppinger, E. C., and Burwell, S. C.: J. A. M. A. **115**:1262, 1940. Gross.[8a]

10. Robb, G. P., and Steinberg, I.: J. A. M. A. **114**:474, 1940.

11. Hubbard, J. P.; Emerson, P. W., and Green, H.: New England J. Med. **221**:481, 1939. Shapiro, M, J.; Keys, A., and Violante, A.: Internat. Clin. **4**: 148, 1941.

12. Touroff, A. S. W., and Vesell, H.: J. Thoracic Surg. **10**:59, 1940. Touroff, A. S. W.; Vesell, H., and Chasnoff, J.: J. A. M. A. **118**:890, 1942.

The electrocardiographic tracing was normal. A roentgenogram revealed "generalized intensification of the hilar and pulmonary vascular markings compatible with cardiac pathology." A diagnosis of patent ductus arteriosus with superimposed Str. viridans infection was made, and the patient was operated on. Uncontrollable hemorrhage occurred during the operation, and death ensued while the patient was on the operating table.

Necropsy showed a persistent ductus arteriosus, which measured 0.6 cm. in length along the superior border and 1.8 cm. along the inferior border. The outside diameter was 1.2 cm., while the inside was 0.6 cm. The ductus arose from the superior and anterior surface of the origin of the left pulmonary artery and entered the arch 0.8 cm. distal to the origin of the left subclavian artery. The entire ductus and the adjoining aorta were completely calcified. The wall of the ductus in its midlateral surface was lacerated over an area about 0.2 cm. in diameter, thus giving a direct communication between its lumen and the left pleural cavity. The intima of the ductus was roughened with atherosclerotic plaques. Its pulmonary side, in addition, contained numerous, large verrucae measuring as much as 0.6 cm. in diameter. Vegetations were also present in the main pulmonary artery. There were none in the arch of the aorta. The pulmonary artery was fusiformly dilated, measuring 4.8 cm. in diameter. Its wall was only 0.1 cm. thick. There was relative right-sided cardiac hypertrophy, the myocardium measuring 0.8 cm. in thickness. The lower lobe of the left lung contained several hemorrhagic infarcts measuring as much as 1.5 cm. in diameter. The spleen weighed 460 Gm.

Case 5.—A woman 23 years of age was admitted, complaining of cough, fever and night sweats of two months' duration and of pain in the shoulder of two days' duration. She had been in poor health since the birth of a child six months previously. Physical examination revealed slight cyanosis of the lips, impaired percussion over the upper part of the chest on the left side, enlargement of the heart to the left, a thrill and a to and fro murmur over the pulmonic area, a slight fever and a blood culture positive for Str. viridans. The electrocardiogram was within normal limits. A roentgenogram showed transverse cardiac enlargement and an increase in pulmonary markings.

Necropsy showed a patent ductus arteriosus, from the lumen of which there protruded a large cauliflower-like vegetation, 2.5 cm. in diameter. The pulmonary artery was greatly dilated. The aortic arch was normal except for a large cone-shaped depression at the entrance of the ductus arteriosus. There were multiple red and gray pulmonary infarcts. The heart was uniformly hypertrophied, weighing 580 Gm.

Case 6.—A white woman 24 years of age was admitted to the hospital for a cesarian section. She had a generally contracted pelvis and was known to have had heart disease for years. Physical examination revealed cardiac enlargement and an accentuated second pulmonic sound. An electrocardiogram showed right axis deviation. A roentgenogram disclosed a prominent pulmonary conus with calcification and right ventricular hypertrophy. Three days after the operation the patient became dyspneic and cyanotic and died.

At necropsy there was found a short, stubby, patent ductus arteriosus, measuring 0.4 cm. in length and 1.9 cm. in outside diameter. It was not calcified. It entered the left pulmonary artery 2.8 cm. distal to the origin of the main pulmonary vessel. Just to the left of the ductal entrance the origin of the left pulmonary artery contained a completely calcified saccular aneurysm, 2.5 cm. in diameter. The pulmonary artery was fusiformly dilated, measuring 3.2 cm. in diameter. The heart showed marked relative right-sided hypertrophy.

CASE 7.—A white woman 21 years of age was admitted, complaining of shortness of breath of three months' duration. During this time she intermittently resorted to rest in bed in order to carry on her daily activities. She was known to have had heart disease all her life. Physical examination revealed slight cyanosis of the lips and face, venous pulsation in the neck, cardiac enlargement to the left, a continuous murmur over the pulmonic area and an apical presystolic murmur transmitted to the axilla. An electrocardiogram showed marked right axis deviation. A roentgenogram revealed a prominent pulmonary conus, an enlarged heart and chronic congestive changes in both lungs. A diagnosis of patent ductus arteriosus was made and operation advised. The induction of anesthesia with nitrous oxide and oxygen was begun, but the patient died before the operation was started.

Necropsy disclosed a patent ductus arteriosus, 1 cm. long and 1.2 cm. in diameter. It entered the main pulmonary artery 4 cm. from the latter's base. There was no calcification, nor were there any vegetations. The pulmonary artery was fusiformly dilated, measuring 3.2 cm. in diameter. The heart weighed 500 Gm. It was globular in shape. The right ventricular wall was as thick as the left. There was considerable mitral fibrosis, with stenosis, but no evidence of active endocarditis.

Posterior Right Subclavian Artery.—Although occurring once in every 60 persons,[13] the posterior right subclavian artery rarely produces signs and symptoms. Dysphagia, inequality in radial pulses, pressure on the thoracic duct and trophic changes in the upper extremity have all been described.[14] The prognosis is good. Treatment usually is unnecessary, but when symptoms do develop, there is always the remote possibility of surgical ligation of the vessel.[15]

CASE 8.—A white boy 3 weeks old was admitted with a history of failure to gain weight and of cyanosis when straining or crying. Physical examination showed an undernourished infant with marked cyanosis. The cardiac rate was rapid, and a systolic murmur was heard over the sternum at the second intercostal space. A roentgenogram revealed a cervical rib on the right side and fused fourth and fifth ribs posteriorly.

Necropsy revealed an enlarged heart. There was only one atrium, and it opened into the left ventricle. The right ventricle was very small, and the pulmonary artery was markedly hypoplastic. The upper portion of the interventricular septum was defective. There was no ductus arteriosus. The aortic arch was normal in position. The innominate artery was absent, having been replaced by the right common carotid artery. The remaining branches were the left common carotid, the left subclavian and the right subclavian. The latter arose opposite the left subclavian artery and then proceeded to the left of and posterior to the esophagus to reach its usual destination on the right side (fig. 2). The esophagus was grooved by the vessel. The right recurrent laryngeal nerve was replaced by branches passing directly to the larynx.

CASE 9.—A girl 8 hours old was admitted because of cyanosis and noisy breathing. Physical examination showed marked cyanosis, laryngeal stridor and slow

13. Goldbloom, A. A.: Surg., Gynec. & Obst. **34**:378, 1922.

14. Anson, B. J.: Surg., Gynec. & Obst. **62**:708, 1936.

15. Cairney, J.: J. Anat. **59**:265, 1924-1925.

respirations. The heart sounds were regular. Tracheotomy was performed because of the stridor, but the infant died.

Necropsy showed a normal aortic arch. The main branches from right to left were the right common carotid, the left common carotid, the left subclavian and the right subclavian. The latter crossed the midline between the esophagus and the vertebral bodies. The ductus arteriosus and the foramen ovale were patent. There were no other cardiac defects. Examination of the head revealed no intracranial hemorrhage. The right recurrent laryngeal nerve was replaced by branches going directly to the larynx. The infant also possessed a cleft palate, an extra finger, a double vagina and an imperfect bicornuate uterus.

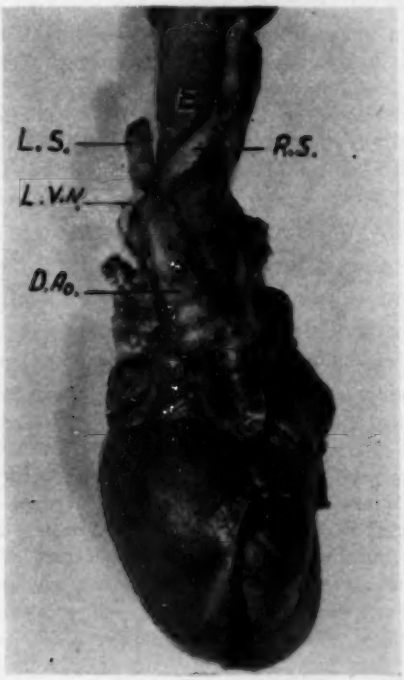

Fig. 2.—Posterior aspect showing the right subclavian artery, *R.S.*, arising from the arch opposite the left subclavian artery, *L.S.* *D.Ao.* is the descending aorta, *L.V.N.* the left vagus nerve and *E*, the esophagus.

Right-Sided Aortic Arch.—The right-sided aortic arch is often asymptomatic until later life, when dilatation and tortuosity of the aorta may cause pressure on the upper mediastinal structures, with resulting dysphagia, dyspnea and laryngeal paralysis.[16] Except for suprasternal pulsation, physical examination usually discloses no abnormality.[17]

16. (*a*) Sprague, H. B.; Frnlund, C. H., and Albright, F.: New England J. Med. **209**:679, 1933. (*b*) Bedford, D. E., and Parkinson, J.: Brit. J. Radiol. **9**: 776, 1936.

17. Brigham, R. O.: Ohio State M. J. **18**:484, 1922.

Bronchoscopic and esophagoscopic inspection reveal a pulsating tumor mass on the right side.[18] Roentgenographically there are widening of the upper part of the mediastinal shadow to the right, pulsation of the aorta in the same location, concave depression of the right and posterior surface of a barium sulfate–filled esophagus and, on intravenous injection of diodrast, presence of the shadow of the arch of the aorta to the right of the trachea.[19] The prognosis is good. Treatment is usually unnecessary. In patients presenting symptoms of pressure caused by obliteration of the ductus arteriosus, the obstruction may possibly be relieved by surgical means.[20]

Case 10.—A white woman 63 years of age was known to have had diabetes for years and had no symptoms referable to the heart or the upper part of the mediastinum. The trachea was in the midline, and the blood pressure varied from 130 to 165 systolic and from 60 to 120 diastolic.

Necropsy revealed an aortic arch passing to the right of the trachea and esophagus and then crossing to the left side at the level of the sixth dorsal vertebra (fig. 3). The left common carotid artery was the first branch. It crossed anterior to the trachea. The next branches were the right common carotid and the right subclavian. There was a persistent posterior aortic root, 2 cm. long, which crossed behind the esophagus. From its tip arose the left subclavian artery. The ligamentum arteriosum measured 2.4 by 0.3 cm. It connected the pulmonary artery with the tip of the left aortic root. The right and the left vagus and the recurrent laryngeal nerves were disposed in a symmetric manner about the right aortic arch and the ligamentum arteriosum, respectively. The anterior portion of the left aortic arch was entirely absent.

Double Aortic Arch.—The double aortic arch is the least common of the anomalies. In 1936 Blincoe, Lowance and Venable [21] collected from the literature a total of 40 cases. Although adults with this condition as a rule are asymptomatic, infants present highly characteristic signs and symptoms. As outlined by Wolman,[22] these consist of "stridulous breathing, chronic cough, respiratory distress while feeding, failure to thrive, susceptibility to pneumonia, head retraction and malnutrition." Physical examination as a rule reveals nothing characteristic. Bronchoscopic inspection may or may not disclose a constriction of the trachea.[23] In infants, a roentgenogram shows a widening of the superior mediastinum, interpreted almost invariably as a persistent or enlarged thymus. It has been stated that a barium sulfate–filled

18. Garland, L. H.: Am. J. Roentgenol. **39**:713, 1938.

19 (*a*) Metzger, H. N., and Ostrium, H.: Am. J. Digest. Dis. & Nutrition **6**:32, 1939. (*b*) Roche, U. J.; Steinberg, I., and Robb, G. P.: Arch. Int. Med. **67**:995, 1941.

20. Jex-Blake, A. J.: Lancet **2**:542, 1926. Sprague and others.[16a]

21. Blincoe, H.; Lowance, M. I., and Venable, J.: Anat. Rec. **66**:505, 1936.

22. Wolman, I. J.: J. Pediat. **14**:527, 1939.

23. (*a*) Hermann, W. W.: Arch. Path. **6**:419, 1928. (*b*) Schall, L. A., and Johnson, L. G.: Ann. Otol., Rhin. & Laryng. **49**:1055, 1940.

esophagus shows a uniform constriction at the level of the arch of the aorta, although, as far as I know, this has really never been demonstrated.

Thus far,[24] treatment of patients presenting symptoms has been entirely palliative and without success. If, however, a diagnosis can be made relatively early, it may be possible to sever the arterial constriction surgically.[25] This has never been attempted.

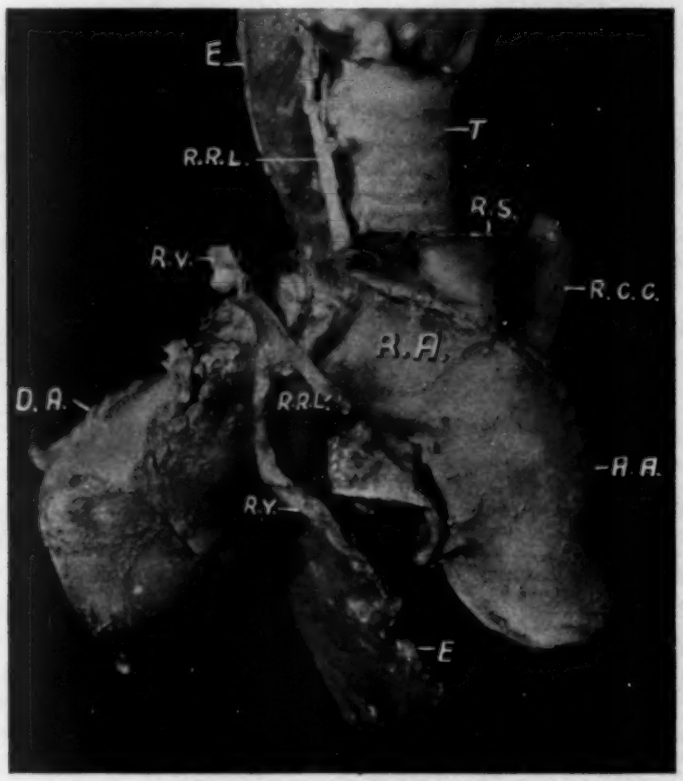

Fig. 3.—Right lateral view showing a right-sided aortic arch, *R.A.* *A.A.* is the ascending aorta, *D.A.* the descending aorta, *R.C.C.* the right common carotid artery, *R.S.* the right subclavian artery, *R.V.* the right vagus nerve, and *R.R.L.* and *R.R.L.'* the right recurrent laryngeal nerve, *E* the esophagus and *T* the trachea.

Case 11.—A Negro man 39 years of age was admitted with symptoms and signs of Addison's disease. On only one occasion, ten months previous to admis-

24. Roche and others.[19b] Wolman.[22] Schall and Johnson.[23b]

25. (*a*) Herbut, P. A., and Smith, T. T.: Arch. Otolaryng. **37**:558, 1943. (*b*) Wolman.[22]

sion, when he exprienced a weak feeling, did he complain of dyspnea. This disappeared with rest in bed. Physical and electrocardiographic examination of the heart indicated that it was normal. A roentgenogram of the chest was not made.

Necropsy showed a complete double aortic arch originating from the upper end of the ascending aorta, encircling the trachea and esophagus and ending in the descending aorta posterior to the esophagus. The right arch measured 1.8 cm. and the left 1.2 cm. in diameter. The main branches arising symmetrically from their corresponding arches were the right and the left common carotid artery and the right and the left subclavian artery. The ligamentum arteriosum was present in its usual position on the left side. Both the right and the left vagus and the recurrent laryngeal nerves were symmetrically disposed around their respective arches. There was no evident constriction of the esophagus, nor was there ulceration of its mucosa. There was tuberculous involvement of each adrenal gland.

Case 12.—A report of this case was published in the April issue of the *Archives of Otolaryngology*.[23a] A white girl 2½ months of age was admitted with a history of attacks of dyspnea since birth. More recently these attacks had been associated with cyanosis and were particularly severe during feeding. Physical examination gave essentially negative results, as did roentgen examination of the chest. In the hospital, several attacks of dyspnea were observed, during which her head was turned to the left, her neck extended and her head flexed. Bronchoscopic examination was attempted but, because of the dyspnea, could not be carried out. Tracheotomy did not relieve the respiratory obstruction, and the child died five days after admission.

Necropsy disclosed a double aortic arch forming a complete arterial collar about the trachea and esophagus (fig. 4). The right arch measured 1.0 cm. and the left 0.5 cm. in diameter. As in the preceding case, the larger vessels arose symmetrically from their corresponding arches. The first branches were the right and the left common carotid, followed by the right and the left subclavian artery. The ductus arteriosus measured 1 cm. in length and 0.4 cm. in diameter. All the vessels were patent. The esophagus was definitely constricted, but its mucosa was not ulcerated. Compression of the trachea could not be satisfactorily demonstrated at postmortem examination. There was no pneumonia.

COMMENT

The disposition of the recurrent laryngeal nerves is worthy of mention. They are normal in cases of coarctation of the aorta and in cases of patent ductus arteriosus. In cases of posterior right subclavian artery the right recurrent laryngeal nerve is usually replaced by branches passing directly from the vagus nerve to the larynx. Occasionally, it winds around the inferior thyroid artery, while at other times it envelops the right vertebral artery.[15] In cases of double and cases of right-sided aortic arch the right recurrent laryngeal nerve passes around the arch instead of around the right subclavian artery. The position of the left recurrent laryngeal nerve varies. It is normal when there is also a left aortic arch or when the ductus arteriosus is on the left side, joining the pulmonary artery with a persistent left aortic root. When there is a

left innominate artery and the ligamentum arteriosum is on the right side, the left recurrent laryngeal nerve winds around the left subclavian artery.[26]

A word should also be added about the intravenous use of diodrast. The technic as perfected by Robb and Steinberg [10] appears to be simple and certainly gives excellent results. Special apparatus, however, is needed, and the operator must be highly skilled. It therefore is not

Fig. 4.—Posterior view showing the left aortic arch, *L.A.A.*, and the right aortic arch, *R.A.A.*, uniting to form the descending aorta, *D.Ao.* *L.S.* is the left subclavian artery, *R.S.* the right subclavian artery, *D.A.* the ductus arteriosus and *E* the esophagus.

practical for the ordinary hospital. The injection of diodrast in itself is not without danger. Robb and Steinberg [10] have made 486 injections in 233 patients, many of whom were seriously ill, and have seen no serious consequences. Occasionally, however, the intravenous use of diodrast in excretory urography has produced death. Nine such cases

26. Sprong, D. H., and Cutler, N. L.: Anat. Rec. **45**:365, 1930.

have been reported in the literature.[27] Doubtless, there are many similar instances which have not been reported. It therefore seems reasonable to suggest that when a positive diagnosis can be made by less dangerous means, such as roentgen study of a barium sulfate–filled esophagus, esophagoscopy and bronchoscopy, intravenous injection of diodrast should not be resorted to. If it is used, the patient should first be tested for sensitivity to iodine.

SUMMARY

Anomalies of the aortic arch may be divided into five separate groups. In approximately the order of frequency the types are: coarctation of the aorta, patent ductus arteriosus, posterior right subclavian artery, right-sided aortic arch and double aortic arch. All of these are easily explained embryologically. For an antemortem diagnosis, bronchoscopic, esophagoscopic and roentgenographic studies are indispensable. Surgical intervention gives excellent results in cases of patent ductus arteriosus. The other anomalies are less amenable to treatment. This is particularly true of a double aortic arch in an infant. All infants presenting symptoms of this anomaly have died of strangulation.

27. Goldburgh, H. L., and Baer, S.: J. A. M. A. **118**:1051, 1942.

Case Reports

DIFFUSE METAPLASTIC GASTRITIS IN A PATIENT WITH PROLONGED CACHEXIA AND MACROCYTIC ANEMIA

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In man and higher mammals a so-called law of specificity of tissue growth postulates that the cells composing a given structure in the fully developed organism are capable of reproducing only identical morphologic and functional types. Exceptions to this rule are encountered in pathologic states and relate principally to the epithelial lining surfaces and the supporting connective tissues of the body. Morbid alterations of epithelial growth in particular have been observed in various organs subject to prolonged inflammation or deprived of adequate nutrition. Thus the tall columnar epithelial cells lining the bronchial tubes not uncommonly acquire a stratified squamous composition under the local stimulus of some chronic bacterial, mechanical or chemical irritation or during the course of certain long-standing dietary deficiencies. Less often, squamous metaplasia is found in the mucosa of the urinary bladder, renal pelvis, ureter, urethra, accessory nasal sinuses, gallbladder, prostatic gland, pancreatic ducts, portions of the gastroenteric canal and elsewhere. When these variants become established in any adult tissue, the process appears largely irreversible, replacement and growth of the altered tissue taking place by reproduction of the acquired type of epithelial cell. It is noteworthy, however, that in certain neoplasms, presumably arising from metaplastic epithelium, both the original and the acquired form of epithelial cell may be observed in the same tumor. Two distinct types of epithelial metamorphosis have been described as involving the mucosal coat of the gastroenteric tract. One of these undoubtedly illustrates heterotopia rather than true metaplasia and proceeds unrelated to local inflammatory or to general deficiency states. This form of epithelium is well developed, functionally active and of wide distribution; typical examples are: bits of gastric mucosa within a diverticulum of Meckel's type, scattered nests of pancreatic glands within the stomach or throughout the small intestine, portions of gastric mucosa indenting the lining coat of the lower part of the esophagus and zones of esophageal mucosal epithelium interpolated between the gastric glands of the cardiac end of the stomach. The other type of epithelial mutation represents true metaplasia. It has been infrequently observed in the colon and rectum and is extremely rare in the gastric mucosa, where it follows severe local inflammatory reactions or complicates prolonged dietary deficiencies. This transformation of the glandular epithelium tends toward a frank squamous cell type but is quite restricted

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in its degree of development, extent and distribution; seldom does it encompass more than a tiny segment of the organ involved.

Diffuse areas of squamous metaplasia, regardless of the causative factors, have hitherto not been described in the gastric mucosa as far as can be determined by an examination of the literature. Likewise, nothing appears to be known of such a condition elsewhere in the intestinal tract. The case under consideration is particularly arresting in that the tissue showing metaplastic changes comprised almost two thirds of the entire lining surface of an unusually small stomach. The nature and the extent of this condition raise several points of interest relating to gastric physiology and general metabolism.

REPORT OF A CASE

A Negro woman 52 years of age was first admitted to the Cincinnati General Hospital Jan. 18, 1935, in a profoundly weak and stuporous condition. It was learned that she had been suffering from intermittent attacks of vomiting and midabdominal pain for several years. These symptoms had increased markedly in severity during the preceding month, forcing her to remain in bed. She stated that in recent weeks the vomiting began immediately after eating food of any kind, or even after drinking water, and was followed by pain, usually localized around the umbilicus. Hematemesis was never present. She was accustomed to take mild laxatives daily. Her loss of weight in the past month was reckoned at 5 to 10 pounds (2.3 to 4.5 Kg.). For two weeks she had also suffered from frequency of urination and dysuria with the passage of some blood. The menopause had occurred twelve years previously. The patient presented no significant positive physical findings other than marked emaciation. Urine obtained by catheter was loaded with leukocytes, and the benzidine test was positive. The blood urea nitrogen amounted to 14 mg., and the blood sugar during fasting to 57 mg., per hundred cubic centimeters. An erythrocyte count made a week after admission was 2,400,000; no remarks regarding the appearance of the cells were recorded. Under a regimen of frequent ingestion of easily assimilable foods, abundant intake of fluids and other supportive measures, the vomiting and abdominal pain subsided; the patient gained strength slowly and showed progressive improvement in general condition but continued to complain of various unrelated and transient symptoms. An intravenous pyelogram made February 15 disclosed marked hydronephrosis on the right. The involved kidney was quite mobile, descending to the level of the first sacral segment from the upper level of the third lumbar body. On the assumption that much of her vague complaints could be relieved by correcting the nephroptosis on the right, the patient was transferred to the surgical service. February 21, nephropexy was performed, with the patient under local anesthesia, and since there were no untoward postoperative incidents, she was discharged March 16, considerably improved, with instructions to return one month later for further observation. There is no record that a Kahn or a Wassermann test was made on the blood during this period of hospitalization. The patient failed to keep her appointment and was not seen again for almost nineteen months.

Oct. 2, 1936, the patient was again brought into the hospital in a semicomatose state, unable to give a coherent account of her condition. On general supportive measures she improved sufficiently to tell her history in some detail ten days later. She stated that her present trouble began six months previously following a day of overexertion at heavy manual labor. The following day she complained of fatigue and vomited several times. She recalled that these vomiting spells had

occurred once every two or three weeks for about three years but could not be attributed to any reasonable cause. During the last six months the vomiting began to occur more frequently. About five months before admission she noted some numbness in her fingers and toes and thereafter perceived a more or less continual tingling sensation in these members. This continued throughout the ensuing months, and about six weeks before hospitalization exertional dyspnea and unsteadiness of gait began to appear. On questioning it was learned that her tongue had been sore for about one year; this she attributed to eating coarse foods. All her teeth had been extracted some time before. Three weeks preceding admission severe watery diarrhea started suddenly, and she passed fifteen to twenty stools daily, which occasionally were tinged with blood. Shortly after this began, she started coughing and expectorated considerable mucopurulent sputum. Feeling extremely weak and apprehensive, she asked to be taken to the hospital. After detailed questioning about her past history, she remembered that she had had typhoid fever as a youngster, and also pneumonia on one occasion, though the dates could not be recalled. Her dietary habits had been irregular, probably for most of her life, and the choice of foods extremely limited. During the past several years she had had $2.50 a week to spend on food; an undetermined portion of this sum was used for cigarets and other items. She maintained, however, that despite her frugal diet, her appetite remained good for foods that she could not afford. The patient had been married but to her knowledge was never pregnant. She learned in 1925 that she had acquired syphilis from her husband. The antisyphilitic treatment recommended by a physician was refused. For a number of years she had subsisted entirely on charity and had lived in squalid surroundings. She drank any type of alcoholic liquor that was available but was apparently limited in its intemperate use by financial circumstances.

The day following her second admission the essential findings were as follows: The temperature was 97 F.; the pulse rate, 89; the respiratory rate, 30; the blood pressure, 85 systolic and 55 diastolic. Emaciation was pronounced. The mucous membranes were pale. The edges and posterior third of her tongue were smooth and the papillae atrophied. Many coarse rales were heard over the entire back. Both knee jerks were absent. Some diminution of vibratory sense was present in the lower parts of both legs, more marked on the right side. Roentgen examination revealed discrete and confluent areas of bronchopneumonia throughout the lower portions of both lungs, most marked at the right base.

The Kahn test of the blood was positive (3 plus). The icteric index was 11. The blood chloride amounted to 524 mg., the blood urea nitrogen to 48 mg. and the blood sugar to 74 mg. per hundred cubic centimeters. The carbon dioxide–combining power was 46 volumes per cent. Stool cultures were negative for typhoid and dysentery bacilli. A dextrose tolerance test gave results within the normal limits. The hemoglobin content was 8 Gm.; the erythrocyte count, 1,950,000; the leukocyte count, 8,950, with polymorphonuclear neutrophils 88 per cent. The red blood cells were enlarged, distorted and well filled with hemoglobin; no immature forms were noted. The specific gravity of the urine was 1.015; there was albumin (plus 1); the leukocytes numbered 50 per low power field; a few erythrocytes and some epithelial cells were found. Gastric analysis after administration of histamine showed no free hydrochloric acid.

In view of the severe macrocytic anemia associated with complete gastric achlorhydria, sore tongue, tingling sensations, numbness and evidence of damage

to the vibratory sense in the extremities, a diagnosis of probable pernicious anemia was made. Sprue was also considered, although examinations of the stools lent little support to this possibility. Supportive treatment was instituted for the bronchopneumonia and the unexplained diarrhea, which rapidly subsided. The patient was given several transfusions of whole blood and large intramuscular injections of liver extract, and in an attempt to restore the weight she had lost, she was placed on a high caloric diet including brewer's yeast. Despite the intensive liver therapy, the reticulocyte count never exceeded 2 to 3 per cent. After two weeks of hospitalization, a cutaneous lesion developed over the left side of the forehead, which was diagnosed as herpes zoster of the ophthalmic branch of the fifth nerve. This subsided uneventfully. The blood urea nitrogen on October 19 amounted to 20 mg. per hundred cubic centimeters; the total serum protein amounted to 8.7 Gm., the serum albumin to 4.6 Gm. and the serum globulin to

Blood Counts and Weight

Date	Hemoglobin, Gm.	Red Blood Cell Count	White Blood Cell Count	Polymorphonuclears	Lymphocytes	Monocytes	Reticulocytes	Body Weight, Lb.	Comment
1/26/35	...	2,400,000	.....	..	..	...	...	....	
10/ 2/36	8.0	1,960,000	8,950	88	10	2.0	0.7	....	Moderate poikilocytosis; red blood cells large and well filled with hemoglobin; no nucleated forms noted. Average corpuscular volume 121 cubic microns. Average corpuscular hemoglobin 47 micromicrograms
10/ 9/36	10.5	2,200,000	10,000	..	..	...	0.5	73.5	
10/12/36	8.0	2,200,000	9,500	65	35	...	0.8	....	
10/19/36	7.2	2,000,000	8,100	74	24	2.0	3.0	69.0	
10/27/36	9.0	1,880,000	.....	..	..	...	1.9	....	
11/ 1/36	9.0	2,660,000	.....	..	..	...	...	70.0	
11/16/36	9.6	2,650,000	.....	..	..	...	...	....	
11/30/36	9.4	4,200,000	13,200	70	29	1.0	...	....	
12/ 3/36	...	.....	.....	..	..	...	...	70.75	
12/27/36	...	4,350,000	26,350	92	5	3.0	...	....	
1/ 5/37	10.0	4,040,000	12,200	79	11	10.0	...	....	
1/29/37	10.0	5,370,000	.....	..	..	...	...	65.0	Sedimentation rate 58 mm. Hematocrit reading 32 per cent
2/19/37	11.5	3,540,000	8,960	..	..	...	...	....	
3/19/37	9.7	3,850,000	.....	..	..	...	...	....	
4/ 8/37	...	.....	.....	..	..	...	...	78.75	
8/11/39	10.5	3,650,000	.....	..	..	...	...	92.0	
9/15/39	....	.....	.....	..	..	...	...	86.5	
11/24/39	....	.....	.....	..	..	...	...	93.75	
10/ 6/40	5.0	1,550,000	4,450	..	..	...	...	....	

4.1 Gm. per hundred cubic centimeters. The following day a Wassermann test of the spinal fluid and a colloidal gold test were reported negative. The blood picture improved steadily, and November 1 the hemoglobin was 9.9 Gm. and the erythrocyte count 2,660,000. Two months later the hemoglobin had reached 10 Gm. and the red blood cell count 4,040,000 (table). Morphologically, the erythrocytes now apparently contained a normal amount of hemoglobin. The basal metabolic rate December 29 was +13 per cent. Roentgenograms of the gastroenteric tract made the following day showed the stomach to be small and contracted, lying almost entirely in the left upper abdominal quadrant. Its walls were smooth and showed no active peristaltic waves. A concentric tubular deformity of the pyloric end was constantly noted (fig. 1). The duodenal bulb was small and led into a dilated second and third portion of the duodenum. When the patient was in an extreme Trendelenburg position, the barium sulfate regurgitated into the esophagus through the cardiac sphincter. The rigidity of the stomach suggested linitis plastica. At the end of twenty-four hours there was 50 per cent gastric retention. A gastroscopic examination Jan. 4, 1937

revealed that the mucosa was pale and the rugae everywhere absent; the pylorus and the antrum were not visualized. The impression at this time was that of atrophic gastritis.

At this time doubts about the original clinical diagnosis were expressed. Because of the absence of immature red cells in the peripheral blood and the failure of the reticulocytes to respond to liver therapy, and in view of the old history of acquired syphilis, the strongly positive Wassermann reaction of the blood and the unusual appearance of the stomach when studied roentgenologically

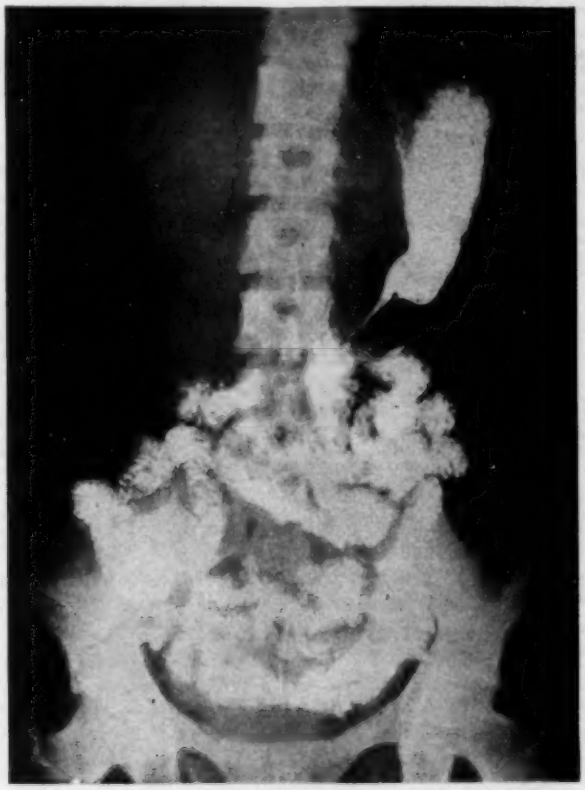

Fig. 1.—A roentgen film taken immediately after a fluoroscopic examination of the stomach. The funnel-shaped narrowing of the stomach appears more prominent over its lower half.

and gastroscopically, a diagnosis of gastric syphilis was held probable. The gastric content, examined repeatedly after injections of histamine, continued to show absence of free hydrochloric acid. The patient was given several intramuscular injections of bismuth subsalicylate and large intravenous doses of sodium iodide. A course of nine intravenous injections was begun with an initial dose of 0.3 Gm. of neoarsphenamine followed by four more of 0.6 Gm. each at weekly intervals; for the last four injections the dose was increased to 0.9 Gm. After the pneumonia had subsided the patient remained afebrile with minor

exceptions for the duration of her hospital course. She was discharged, April 8, as improved, weighing 78¾ pounds (35.8 Kg.), after seven months' hospitalization and was advised to return for further treatment and observation.

She was seen again June 18 and said that her appetite was good and that she felt fine. A gastroenteric series June 22 showed no gastric residue at the end of twenty-four hours but otherwise showed conditions similar to those at the previous examination. The patient neglected to return for observation and was lost sight of for a period of two years. July 3, 1939, she reappeared unexpectedly, saying that she had been quite well until three months before, when she began to lose appetite and to vomit occasionally. She also felt that she was getting weaker and said that she had lost 10 pounds (4.5 Kg.). Her best weight had been 105 pounds (47.6 Kg.), sometime in May 1939. There had been some numbness and tingling of the hands and feet during the past year. At the time she weighed 95 pounds (43 Kg.) and despite complaints her general condition showed considerable improvement over that two years before. Her mucous membranes were slightly pale. Palliative measures were advised for her complaints, and she was told to return a week later. A gastroenteric series was made June 11, which yielded findings essentially the same as those previously noted. Reexamination with the gastroscope September 22 again gave the impression of atrophic gastritis, but the possibility of diffuse fibrosis of the gastric mucosa was considered. When seen again, November 24, she still complained of numbness and tingling of her hands and feet but was otherwise symptom free. Her weight was 93¾ pounds (42.5 Kg.). She was to return in two months but did not appear until Oct. 6, 1940, ten months later.

On that date she was brought to the hospital by ambulance at 1:51 p. m., critically ill and unable to respond to questioning. A friend who accompanied her volunteered the information that she had been steadily growing weaker and during the past few weeks had often complained of abdominal pain. For several days preceding admission she had been unable to void, and weakness obliged her to remain in bed. On admission the bladder was catheterized and 700 cc. of bloody urine withdrawn. The patient was critically ill, emaciated and slightly dehydrated. The temperature was 98 F., the pulse rate 88, the respiratory rate 20 and the blood pressure 80 systolic and 50 diastolic. There was generalized abdominal tenderness to palpation. The mucous membranes were extremely pale. The hemoglobin content was 5 Gm., the red blood cell count 1,550,000 and the leukocyte count 4,450. The blood urea nitrogen amounted to 75 mg. per hundred cubic centimeters. The carbon dioxide-combining power was 30 volumes per cent. An immediate transfusion of 250 cc. of whole blood was given. Her condition became critical, and she died at 3:55 p. m. the following day.

The final impression was one of marked cachexia, probably due to gastric carcinoma; uremia; syphilis (?) of the stomach; marked anemia.

Autopsy (fifty hours after death).—The body, measuring 160 cm. in length, was that of an extremely poorly nourished mulatto woman, appearing approximately the stated age of 57 years. Rigor mortis was well developed in the muscles of the extremities, neck and jaws. There were no external deformities. There was moderate pitting edema of the feet and legs, extending to both knees. The inelastic smooth skin was free from eruptions and scars. The gums and the mucous membrane were pale pink, and the jaws edentulous. Both corneas were cloudy. The panniculus over both the thorax and the abdomen was extremely thin and light yellow. The underlying dark red musculature was thin and poorly developed. The peritoneal cavity contained a moderate amount of thin yellow

fibrinopurulent exudate collected in small areas about the loops of the small intestine. The mesenteric lymph nodes were slightly enlarged. The pleurae were glistening gray except where covered by thin fibrous adhesions. The lining of the pericardial sac was smooth, gray and glistening. There were no external abnormalities about the neck, and the organs here were not removed. The thoracic portion of the esophagus appeared slightly dilated and was filled with light yellow watery material.

The small, light red heart weighed 210 Gm. The endocardial lining was smooth and translucent, and the valve leaflets were thin and free from scarring. Sections through the interventricular septum and the left ventricular wall showed a soft, flabby, pale red musculature. The coronary arteries were thick and the intimal surfaces covered by irregular patches of opaque, brittle, yellow material. The inelastic, nontortuous abdominal aorta presented many irregular raised opaque yellow intimal plaques. A few of these were ulcerated.

The lungs, right and left, weighed 700 and 450 Gm., respectively. All the lobes were rather poorly aerated and exuded moderate amounts of dark red watery fluid on pressure. No areas of consolidation were present. The hilar lymph nodes were slightly enlarged and presented black pigment.

The reddish brown spleen weighed 175 Gm.; it was surrounded by thick fibrous adhesions which were broken with difficulty. The splenic substance was dull pink and friable, with moderately accentuated follicular markings.

The pale red liver weighed 1,400 Gm. and was covered by a thin translucent capsule except for a few small thickened areas. The liver tissue was pale reddish brown, soft, friable, slightly greasy. The gallbladder was filled with approximately 75 cc. of greenish black bile and had a dark greenish black velvety mucosal lining. The biliary passages were patent.

The pancreas appeared normal. The stomach was extremely small, measuring 26 cm. along the greater curvature from the esophageal junction to the pyloric ring (fig. 2). Its outline was fairly regular, although the reduction in size appeared slightly more pronounced over the lower half. While the caliber of the pylorus was uniformly reduced, there was no evidence of any obstruction. The wall was markedly thickened and cut with a gritty sound. The rugae were everywhere flattened and rarely visible, the mucosal coat dull gray and lusterless. The valvulae conniventes of the duodenum and jejunum were distinct. The esophagus appeared slightly dilated, its mucosa smooth and gray. No abnormalities were present at the cardioesophageal junction. The mucosa and wall of the colon showed no noteworthy changes. The peritoneum covering many of the lower loops of small intestine was thick, lusterless and coated by a small amount of thick yellow fibrinous exudate.

The two equal-sized kidneys together weighed 225 Gm. Their stripped surfaces were pale grayish red and nongranular. Coronal sections showed cortices and medullae quite well defined, with occasional slender gray streaks extending from the medullary tips well into the cortex. The pelves appeared normal in size, and their mucosal lining was dusky reddish gray. The ureters possessed smooth thin glistening white linings.

The urinary bladder contained approximately 500 cc. of bright red urine. The bladder wall was thickened, dark red and extremely friable; the mucosal surface presented numerous large, dark red ulcerated areas. Thick shaggy fibrinous exudate covered the serosal surface.

The ovaries and oviducts were uniformly reduced in size. The atrophic uterus contained a round intramural myoma, 3 cm. in diameter, in the fundus.

The adrenal glands appeared normal in size, shape and consistency for a patient of this age.

The brain was not examined because of restrictions on the autopsy.

Anatomic Diagnosis.—Acute diffuse gangrenous cystitis with pelvic and early generalized peritonitis; bilateral ascending pyelonephritis; pulmonary congestion and edema; marked atrophy and fibrosis of the stomach; pleural adhesions; chronic perisplenitis; acute splenitis; fatty(?) infiltration of the liver; leiomyoma uteri; marked cachexia.

Microscopic Examination.—Sections from the midportion of the esophagus showed an irregular thinning of the stratified squamous epithelium as though portions of the more superficial layers had sloughed away. Minor degenerative

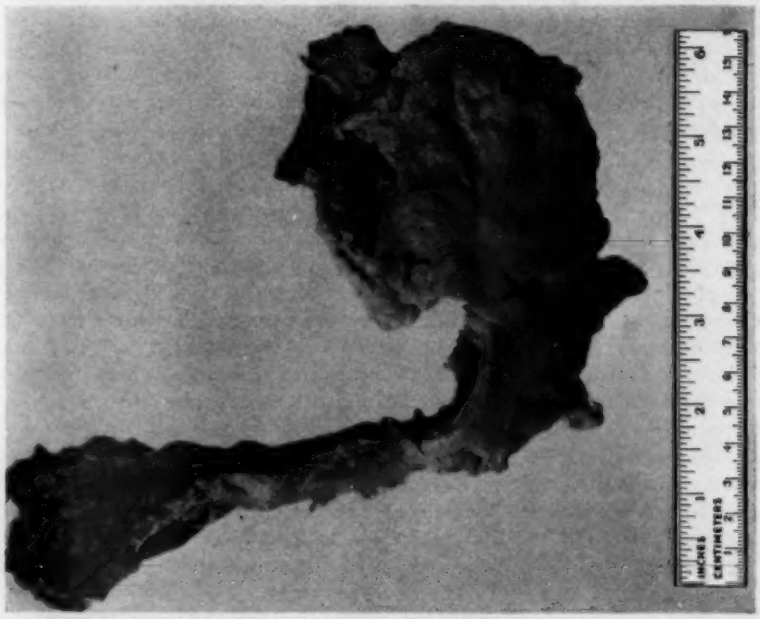

Fig. 2.—Stomach as removed at autopsy after excision of a portion on the lesser curvature for histologic study. The cross indicates the approximate region opposite that on the posterior wall from which the tissue shown in figure 3 was taken.

changes were present within the epithelial cells, but inflammatory cells were absent. In the submucosa, small collections of chronic inflammatory cells were scattered about the engorged capillaries and about the mucous glands. Throughout this coat the veins were considerably dilated. Some slight old scarring was present. The muscular coats appeared normal. In portions of the esophagus bordering on the cardia of the stomach, groups of large and small lymphocytes, clasmatocytes and polymorphonuclear leukocytes extended in tonguelike projections from the subepithelial layers well into the underlying muscle coat; here some patchy fibrosis was also present. Sections from the lesser curvature of the stomach immediately below the esophageal junction showed the gastric glands

considerably distorted, atrophied and partly replaced by condensed stromal connective tissue; there was interstitial infiltration by a considerable number of lymphocytes, large mononuclear cells, polymorphonuclear neutrophils and eosinophils. The destruction of the glandular elements increased in the descent along the lesser curvature, and they were completely replaced by stratified squamous epithelium about 1.5 cm. below the esophageal junction. The stroma beneath the new lining epithelium was also infiltrated with chronic inflammatory cells and thrown up into small papillary projections. Sections removed from the greater curvature of the fundus showed the gastric glands and columnar lining cells entirely absent. The mucosa was represented by a continuation of the stratified squamous epithelium covering the esophagus, with varying amounts of chronic inflammatory cells infiltrating the subepithelial tissue. In order to appreciate the extent of these changes fully, a continuous section almost 1 cm. in width was made from the posterior wall of the stomach about 2 cm. above the greater curvature, extending from the cardioesophageal junction through the pyloric ring. This was blocked in paraffin, and several large sections were cut on a special microtome at about 10 microns in thickness and mounted in the routine manner. This yielded full information on the typography and the extent of the changes in various portions of the wall. Stratified squamous lining epithelium was found to extend along this zone from the esophageal junction to within 7 cm. of the pyloric ring. Another smaller section taken from the pyloric region along the greater curvature showed the stratified squamous epithelium reaching to within 3 cm. of the pyloric valve. Throughout all sections striking hypertrophy and fusing of the fibers of the muscularis mucosae were observed, increasing the thickness of this coat in most areas from six to ten or more times its normal diameter. Within this structure were large, irregular bands of hyalinized connective tissue, which also contributed considerably to its increased width. The middle circular muscle coat showed uniform hypertrophy, but this appeared proportionately less marked than that of the muscularis mucosae (fig. 3). Irregularly placed along the deeper layers of the muscularis mucosae were compact aggregates of small lymphocytes surrounding the small blood vessels. Lying parallel to the long axis of the altered mucosal coat were scattered streaklike collections of large and small lymphocytes, polymorphonuclear neutrophils and eosinophils, with occasional plasma and large mononuclear cells. These subepithelial groups of cells penetrated short distances into the superficial portions of the thickened muscularis mucosae. Here and there bits of the squamous mucosal surface were destroyed by an acute suppurative process. Rarely a small round area of necrosis could be found in the muscularis mucosae beneath and touching the cellular zone. These areas were surrounded by a broken thin layer of epithelioid cells. No remarkable changes were present in either the small or the large blood vessels throughout the stomach. The thickness of the stratified squamous epithelial lining surface varied almost continually and frequently was elevated by small buds of supporting connective tissue stroma infiltrated with chronic inflammatory cells. In some zones it maintained a uniform thickness for a distance of several centimeters (fig. 4*A*), while in an area close by there were alternate high and low zones (fig. 4*B*). At the pyloric antrum there was again present a continuous glandular gastric mucosa. The glands, however, were observed to be severely infiltrated by large and small lymphocytes, mononuclear cells and occasional polymorphonuclear leukocytes. In a few areas they were partially replaced by small strands of fibrous connective tissue. An occasional group of well preserved parietal cells lined portions of the less severely involved glands.

Fig. 3.—Section taken through an area on the posterior wall, 14 cm. above the pyloric ring; hematoxylin and eosin stain; × 25. Note the replacement of normal mucosa by stratified squamous epithelium and the marked thickening of the muscularis mucosae, with presence of inflammatory exudate.

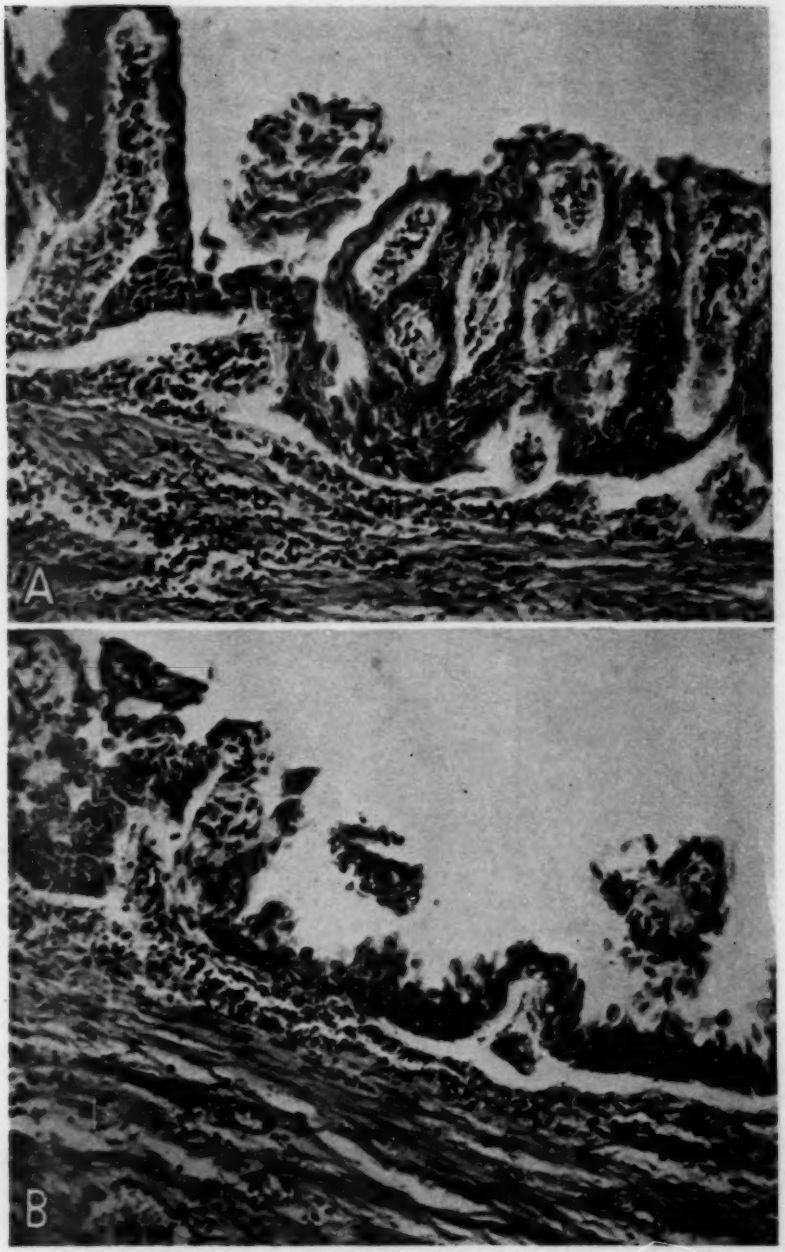

Fig. 4.—*A*, uniformly thick lining of stratified squamous epithelium; hematoxylin and eosin stain; × 160. *B*, alternating high and low portions of the altered mucosa and prominent thickening of the muscularis mucosae; hematoxylin and eosin stain; × 160.

The great bulk of the cells remaining were, however, of the clear cell type. A few slight nonspecific chronic inflammatory changes were present in the superficial portion of the duodenal mucosa.

In the kidneys, rather broad streaks of polymorphonuclear neutrophils extended through the medullary pyramids from the mucosa of the calices well into the cortex. Large areas of tubular epithelium were destroyed, and clumps of cells filled the lumens. Some bits of necrotic tissue contained clumps of gram-positive cocci. There was moderate sclerosis of the small arterioles but relatively slight glomerular damage.

The urinary bladder showed extensive deep acute ulcers of the mucosa, covered with fibrinopurulent exudate. In many instances the necrotic bases of an ulcer penetrated into the outer layer of the muscle coat. In the exudate morphologically resembling staphylococci were clumps of gram-positive cocci. In a few zones the muscle coat was entirely necrotic and showed little cellular inflammatory reaction.

The liver contained in the midzonal portions of its lobules occasional minute foci of acute necrosis of hepatic cells surrounded by polymorphonuclear neutrophils. No other noteworthy changes were present.

Apart from a small amount of acute inflammatory exudate on the serosa of the duodenum, jejunum and ileum, no remarkable changes were present in the small intestine.

In the spleen there was marked infiltration of the red pulp by large mononuclear cells and occasional polymorphonuclears. A solitary small circumscribed fibrous nodule was present in the pulp.

The heart revealed moderate sclerosis of the coronary arterioles, with patchy stellate areas of fibrosis replacing some muscle bundles.

The thoracic aorta had moderate intimal atherosclerotic changes and slight nuclear "dropping" in the medial coat.

The pancreas showed moderate sclerosis of the arterioles and some increase in the interstitial supporting connective tissue.

The lungs disclosed chronic emphysema with some intra-alveolar edema and chronic passive congestive changes throughout all sections.

The sinusoids of the hilar lymph nodes were packed with large coal-pigment-laden mononuclear cells and clumps of polymorphonuclear neutrophils. One section showed a large dense nodule composed of compact collagenous fibers. This contained some anthracotic pigment. Nonspecific reacting cells surrounded the nodule.

Microscopic Diagnosis.—Diffuse gangrenous cystitis with pelvic and generalized peritonitis; bilateral ascending acute suppurative pyelonephritis with abscess formation; chronic diffuse metaplastic gastritis with hypertrophy of the muscle coats; acute focal necrosis of the liver; healed tuberculous(?) hilar lymphadenitis; healed tubercle in the spleen; acute splenitis; slight myocardial fibrosis; slight pulmonary edema; chronic pulmonary emphysema; interstitial fibrosis of the pancreas; leiomyoma uteri; atherosclerosis of the aorta; moderate generalized arteriosclerosis and arteriolosclerosis.

COMMENT

The etiologic agent or the combination of factors effecting the remarkable transformation observed in the inner coats of this uniformly small stomach eludes easy identification. The influence of these changes on the general nutritional state and the blood picture is far more readily understood. Small areas of gastric mucosa containing chronically

inflamed glands at the esophageal junction on the lesser curvature and larger zones of similar mucosa clothing the pyloric antrum indicate the acquired nature and the long duration of the principal process. The fact that the deformed appearance of the stomach observed on roentgenologic examination remained unchanged over a period of more than four years supports this assumption. Histologically the lesion exhibited chronicity but lacked any specific character. There was nothing to suggest syphilis in or about the blood vessels or in the exudative or the productive inflammatory reactions. Special staining for both spirochetes and tubercle bacilli yielded negative results. Furthermore, no other organ examined disclosed specific lesions of syphilis or of active tuberculosis. The production of metaplastic epithelial changes in the stomach by immoderate use of alcohol over an extended period was not substantiated by any history of continual alcoholic abuse, nor do the changes resemble those seen in advanced alcoholic gastritis. The possibility that the patient might have ingested some cauterizing substance destroying a large portion of the gastric lining, or that there had been lengthy exposure of the stomach to the effects of roentgen rays, is unsupported by the history. What is more, stomachs of patients who have survived such episodes for a considerable length of time do not show extensive metaplasias of this type. In the absence of evidence of any of these or similar injuries some etiologic factor other than that relating to direct contact must be sought. The long history of a deficient diet and the clinically observed chronic malnutrition and anemia may be of significance in this regard. While the patient had complained occasionally of sore tongue, and atrophic changes had been noted involving the mucosa over the edges and posterior third of the tongue, a history of dysphagia was never elicited, and studies of the blood failed to reveal the type of simple hypochromic anemia usually observed in the Plummer-Vinson syndrome.[1] On the other hand, a long-standing lack of vitamin A–containing food substances conceivably could have altered the growth of the gastric epithelium, initiating early metaplastic changes, and at the same time lowered the resistance of this tissue to infection. Thus a vicious cycle would be instituted, each extension of the inflammatory response to infection reacting on the already damaged gastric mucosal cells and aiding the development of further metaplastic changes, and this in turn impairing nutritional absorption and favoring additional bacterial penetration and inflammation. The increase in thickness of the muscularis mucosae and its scarification by hyalinized connective tissue seem to define the innermost boundary of the preceding inflammation. The increased thickness of the circular muscle coat possibly represents a work hypertrophy of the imperfectly functioning organ. No obstructive mechanism was found responsible for the recurrent attacks of vomiting noted clinically. The loss of the intrinsic antianemic factor with widespread damage to the mucosal coat, together with the absence of the extrinsic substance in the diet accounts for the severe macrocytic anemia. The slight changes in the liver observed microscopically appeared to be of extremely minor importance. Doubtless they were secondary to inflammatory foci in the stomach and had no possible bearing on storage of the "X" hemopoietic

1. Hoover, W. B.: New England J. Med. **213**:394, 1935.

factor in the liver. Should the assumption be correct that chronic lack of dietary vitamin A was instrumental in producing the primary changes in the gastric mucosa, it still fails to explain the absence of other more common clinical manifestations of this deficiency state; nor does it account for the lack of signs and symptoms of other avitaminoses which must have existed to some extent in chronic starvation of this type, unless it is assumed that the supply of these substances was adequate to prevent clinical manifestations. The gradual but definite improvement in the patient's blood picture and general body nutrition on a selected hospital diet indicate that sufficient absorption was maintained through the duodenal and jejunal mucosal coats to overcome the loss of intrinsic factor in the lower portion of the stomach, a fact substantiated by the absence of noteworthy histologic alterations in the mucous membranes of the duodenum and jejunum.

A final solution to the cause and significance of the changes noted in the stomach of this patient appears to rest on future investigation.

SUMMARY

A Negro woman in the fifth decade of life had, during six years, recurrent attacks of vomiting, abdominal pain and progressive general weakness. There was marked cachexia associated with macrocytic anemia which did not respond to liver therapy. Roentgen examinations showed an extremely small and sluggish stomach. Gastroscopically, the mucosa was pale and rugae were absent. At autopsy the stomach was found to be uniformly reduced in size and its wall regularly thickened with diffuse squamous metaplasia of the mucosa. No apparent cause for the gastric changes was found. The possibility of vitamin A deficiency must be considered.

FOCAL LIPID GRANULOMATOSIS OF THE LUNG FOLLOWING INSTILLATION OF IODIZED POPPYSEED OIL

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Iodized oil is introduced into the bronchial tree for diagnostic purposes on the assumption that it produces no local reaction, is systemically harmless and is readily disposed of by the body. Clinical experience and some experimental work tend to justify the procedure. However, some contrary evidence exists. Bezançon, Delarue and Valet-Bellot,[1] in a study of lungs from patients dying two days, twelve days and six years after instillation of iodized poppyseed oil demonstrated residual oil but no striking tissue response other than the presence of lipophages. That iodized poppyseed oil can produce a significant reaction in the human lung has been demonstrated, though rarely. Rabinovitch and Lederer [2] "saw such a case following the intratracheal introduction of this substance [iodized poppyseed oil] in an old person who later died of bronchopneumonia. The lungs showed changes similar to those seen in lipoid pneumonia." Unfortunately, these authors gave no further description of the case. Lenk and Haslinger,[3] also without giving any pathologic details, have written of the occurrence of "foreign body pneumonia" following the use of iodized oil. The acute reactions described by Archibald and Brown [4] in the early days of bronchography with radiopaque substances undoubtedly have no relation to lipoid pneumonia. The only carefully described case with a true tissue reaction ascribed to iodized oil is that of Wright [5]: Twelve months before the patient's death such oil (rapeseed oil plus iodine) was instilled. One of the main lateral branches of the right bronchus was seen to be blocked completely, but the oil passed readily into the lower branches. Roentgenograms taken a week before death showed the oil still present in bronchiectatic cavities in the neighborhood of a relatively radiopaque mass, especially in parts caudal thereto. A tumor, diagnosed as carcinoma, had occluded the main bronchus. Microscopically, fat was found in macrophages. No collagen formation could be demonstrated with the Van Gieson stain. However, a peculiar pleural reaction was found in the neighborhood, which Wright attributed to the oil. Freiman, Engelberg and Merrit,[6] commenting on this case, suggested that the

From the Department of Laboratories, Beth Israel Hospital.

1. Bezançon, F.; Delarue, J., and Valet-Bellot, M.: Ann. d'anat. path. **12**: 229, 1935.

2. Rabinovitch, J., and Lederer, M.: Arch. Path. **17**:160, 1934.

3. Lenk, R., and Haslinger, F.: Klin. Wchnschr. **4**:1533, 1925.

4. Archibald, E., and Brown, A. L.: J. A. M. A. **88**:1310, 1927.

5. Wright, R. D.: Am. J. Path. **11**:497, 1935.

6. Freiman, D. G.; Engelberg, H., and Merrit, W. H.: Arch. Int. Med. **66**:11, 1940.

picture was that of "the normal walling off of a foreign body that cannot be removed rather than a reaction attributable to the chemical nature of the oil." They suggested that the oil was trapped by the bronchial neoplasm and so the normal removal mechanism was interfered with.

Experiments with animals have vindicated the clinical belief that iodized vegetable oils are relatively innocuous when introduced into the lung. Fried and Whitaker [7] injected iodized oil into the tracheas of cats. They found it well tolerated in amounts up to 1.5 cc. per kilogram. The oil remained in the lungs for long periods, producing only a phagocytic response, without any sclerotic changes. Animals with "snuffles" rid themselves of the oil more rapidly. Pinkerton,[8] using puppies and rabbits, also concluded that iodized vegetable oils were relatively bland. Brown,[9] with cats, also showed that iodized oil stimulated only a phagocytic response, the phagocytes removing the oil along lymphatic channels. Adams,[10] in the discussion of a paper of Cannon's on lipoid pneumonia, mentioned experiments on dogs in which iodized poppyseed oil was introduced intratracheally. This produced local scarring. He commented that in man the oil remained for a considerable time, and that it is not known why sometimes inflammatory reactions occur and sometimes not. Paterson,[11] in an extensive study of the response of lung tissue to oily substances, concluded that poppyseed oil with 40 per cent iodine called forth little reaction. Macrophages took on a finely granular appearance after phagocytosis of the oil, though the amount of phagocytosis was slight. He concluded that in human beings the oil is probably almost completely removed by expectoration. He stated, without giving any of the detailed evidence, that in experimental animals given injections of the aforementioned oil tiny areas of reaction were found after seventy days. Peiper and Klose [12] injected 2 and 3 cc. of 20 per cent iodized sesame oil into 2 guinea pigs. One animal died in twenty-four hours with severe tracheitis and pneumonia; the second was killed after nine days and was found to have severe pneumonia. The histologic details were not described.

I have had the opportunity of studying a lung removed because of a bronchial neoplasm shortly after a diagnostic bronchographic procedure in which iodized poppyseed oil had been instilled into the lung.

A man aged 52 began to have a chronic cough. A relative who was a physician noted the rapid development of clubbing of the fingers. At bronchographic examination 10 cc. of iodized poppyseed oil 40 per cent was used, and an attempt was made to get into the upper lobe of the right lung. The orifice was blocked by a tumor mass, and all of the oil ran into the lower and the middle lobe of the right lung. A few days later, bronchoscopic biopsy revealed squamous cell carcinoma. One month after the initial bronchographic procedure, the right lung was resected. Corresponding to the clinical findings, a neoplasm, diagnosed as

7. Fried, B. M., and Whitaker, L. R.: Arch. Int. Med. **40**:726, 1927.
8. Pinkerton, H.: Arch. Path. **5**:380, 1928.
9. Brown, A. L.: Surg., Gynec. & Obst. **46**:597, 1928.
10. Adams, W. E., in discussion on Cannon, P. R.: Arch. Path. **19**:135, 1935.
11. Paterson, J. L. H.: J. Path. & Bact. **46**:151, 1938.
12. Peiper, H., and Klose, H.: Arch. f. klin. Chir. **134**:303, 1925.

squamous cell carcinoma, partly occluded the right main bronchus. When, immediately after removal, the surgical specimen was examined, numerous small firm nodules, then misinterpreted in the gross as metastases, were found scattered beneath the pleura and in the parenchyma of the middle and lower lobes. The patient had a stormy postoperative course and succumbed to an infection of the wound on the sixth postoperative day. At autopsy, no tumor metastases were found, and the left lung showed no foci similar to those previously seen in the middle and lower lobes of the right lung.

A detailed description of the surgically removed right lung follows.

The lung showed, in addition to the usual three lobes, an azygos lobe. The azygos, the upper and the middle lobe were almost entirely atelectatic (preoperative pneumothorax). On the other hand, the lower lobe was somewhat emphysematous. The mechanism by which this occurred is to be discussed in a separate paper.[13] Since the granulomatous nodules were found in both the atelectatic middle lobe and the emphysematous lower lobe, these factors per se cannot be related to the genesis of the nodules.

In the right main bronchus a polypoid tumor mass, 2 by 1.5 cm., was found close to the cut edge. It projected into the lumen so as to form a valvelike structure partly obstructing the entrance into the lower lobe and, to a lesser extent, that into the middle lobe. In addition, a small submucosal tumor nodule, about 7 mm. in diameter, further incompletely obstructed the lumen of the bronchus to the middle lobe. Another tumor mass, 1.5 by 1 cm., incompletely separated from the aforementioned polypoid mass, totally occluded the orifice of the bronchus to the upper lobe. Continuous with this was a mass about 4 cm. in diameter, involving all the branch bronchi to the upper lobe and also the branch to the azygos lobe, which arose from the bronchus to the upper lobe. At one point the neoplasm invaded the pulmonary vein.

Scattered beneath the pleura of the middle and lower lobes, as well as throughout their parenchyma, were numerous firm brown or gray-brown nodules. These were ovoid or irregularly spherical, some were confluent, and the largest measured about 1 cm. in greatest diameter. They had sharp boundaries and stood up slightly above the cut surface of the lung. The subpleural ones similarly raised the pleura and were visible to the naked eye. They were perhaps slightly more numerous at the apexes of these lobes, but no special distribution could be recognized.

Microscopically, the tumor was seen to be squamous cell carcinoma, with considerable hornification. No tumor was found anywhere but in the major and adjacent subsidiary masses. The invasion of the pulmonary vein was confirmed.

The nodules in the middle and lower lobes consisted of aggregates of tubercle-like structures. At the centers of many were fat globules, appearing as empty spaces in the paraffin sections, staining orange red with sudan IV and black with osmic acid. Stretched over the periphery of the fat droplet in many instances was a giant cell of the foreign body type. Epithelioid cells and macrophages surrounded these, with a peripheral rim of lymphocytes and fibroblasts. Many of the nodules contained no free fat, but the central core was formed by lipophages and epithelioid cells. About the "tubercle" in many instances a fairly dense collar of collagen was laid down. With silver impregration, a fine network of fibers surrounded each tiny focus and bound adjacent ones. No argyrophilic fibers were found in

13. Cohen, A. G.: Personal communication to the author.

the central portions occupied by the lipophages. With Van Gieson and azan carmine stains, fibrous strands were found occasionally crossing an individual unit, and a rare focus appeared entirely replaced by fibrous tissue. Scattered multinucleated giant cells with large fat-containing vacuoles were fairly numerous. In the alveoli adjacent to the involved areas there were clusters of lipophages, and occasional fat-laden macrophages were found in lymphatics at some distance.

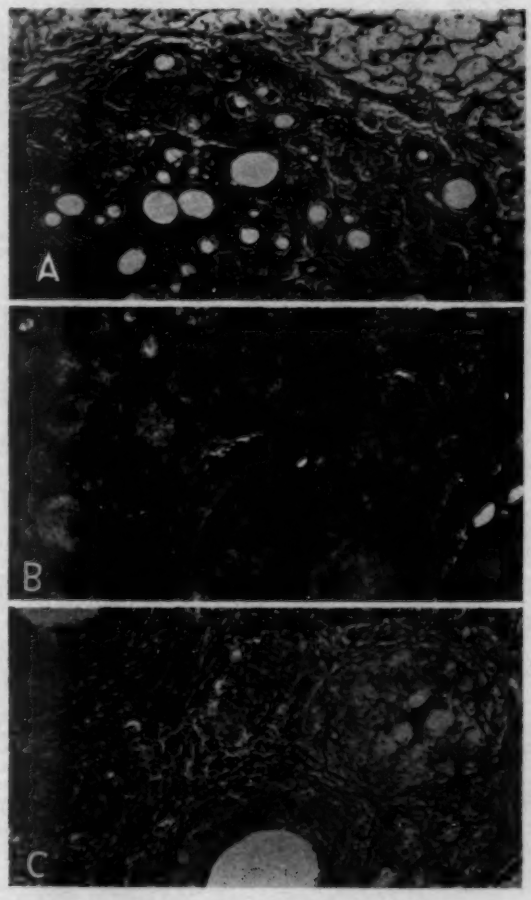

Fig. 1.—*A*, focal granuloma, paraffin section, hematoxylin and eosin stain. *B*, focal granuloma, frozen section, osmic acid stain. *C*, individual tubercle-like structures in granulomatous nodules. Azan carmine stain.

No iron pigment was found in the nodules. Ziehl-Neelsen stain revealed no acid-fast substance such as has been described in cases of cod liver oil pneumonia. However, with this stain, some of the giant cells assumed a slightly pinkish hue, and occasional reddish granules were seen in some of the epithelioid cells. Material preserved in Kaiserling solution, long after the original fixation, was stained with nile blue sulfate; the free fat stained a deep magenta; the intra-

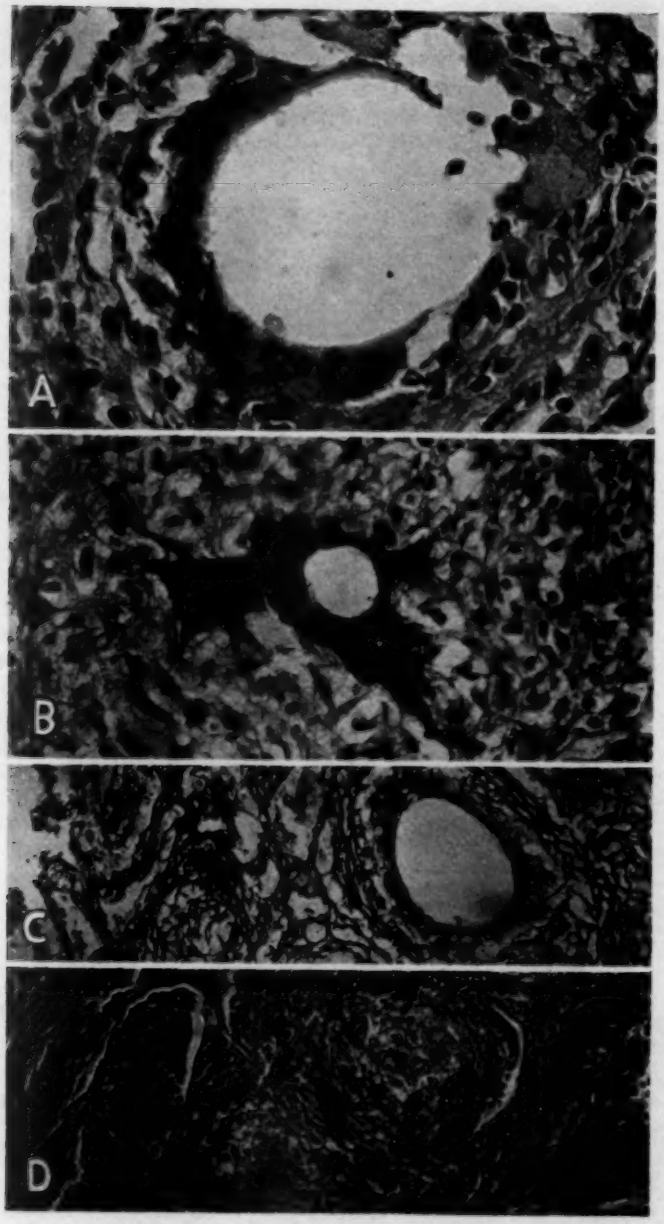

Fig. 2.—*A*, foreign body giant cell stretched over the surface of an oil droplet in the center of a "tubercle." *B*, giant cell containing an oil droplet in its center, surrounded by epithelioid cells and lipophages. Note the similarity to a tubercle. *C*, silver impregnation showing a network of argyrophile fibers about and between "tubercles." *D*, Van Gieson stain showing collagen formation around and between "tubercles."

cellular fat gave a purple-blue color. The interpretation of these reactions after such a long period of fixation seems hazardous. However, the staining would indicate that the free fat was neutral and saturated.

The saturation of the fat (by iodine) is further indicated by the fact that roentgenograms of thin slices showed tiny radiopaque areas that agreed in both distribution and size with the oil droplets seen in microsections. Unfortunately, attempts at extracting the oil and chemically analyzing it were unsuccessful.

The pathologic diagnosis was: squamous cell carcinoma of the main bronchus to the right lung, with occlusion of the bronchus to the upper lobe and partial obstruction of the bronchi to the middle and lower lobes of the lung; atelectasis of the azygos, upper and middle lobes; emphysema of the lower lobe; granulomatosis of the middle and lower lobes (foreign body reaction to iodized poppyseed oil).

COMMENT

It seems clear that in this case there occurred a granulomatous reaction to iodized poppyseed oil instilled into the lung. From the survey of the literature and from general clinical experience, this must represent a most unusual result. The only case which is at all similar is that of Wright,[6] and in that no granulomatous proliferation was found in the pulmonary parenchyma. The 2 cases do have in common the trapping of the iodized oil (partly or completely) behind a bronchial tumor. It may well be that this mechanical factor played a role in these 2 cases. However, in the present case the time elapsing between the instillation of the oil and the examination of the lung was only one month. In many cases, iodized oil has been demonstrated in the lung for longer periods without evidence of a similar reaction. I have examined two other pneumonectomy specimens which had previously received iodized poppyseed oil and found no similar lesions. Some other factor, or factors, must be involved. This case throws no immediate light on what these might be. It should draw attention, however, to the fact that instillation of iodized oil into the human lung cannot be carried out without a certain danger of producing a chronic tissue reaction. The possibility that such foci might be misinterpreted as tumor metastases should be borne in mind.

SUMMARY

A case with multiple foci of lipid granulomatous reaction in a lung resected for carcinoma of the bronchus one month after diagnostic instillation of iodized poppyseed oil is presented. The lesion was restricted to the lobes into which the oil had been introduced and in which the bronchi were partially obstructed by the tumor.

Laboratory Methods and Technical Notes

AN INSTRUMENT FOR REMOVING THE SPINAL CORD THROUGH THE FORAMEN MAGNUM

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The need for routine examination of the spinal cord in complete autopsies led to the development of an instrument which facilitates removing the cord from within the cranial cavity without the necessity of removing the spinal processes, laminas and dura. The instrument now in use is a modification of one described by Brines, Webster and Chesney [1] and was evolved after numerous types of blades had been tried.

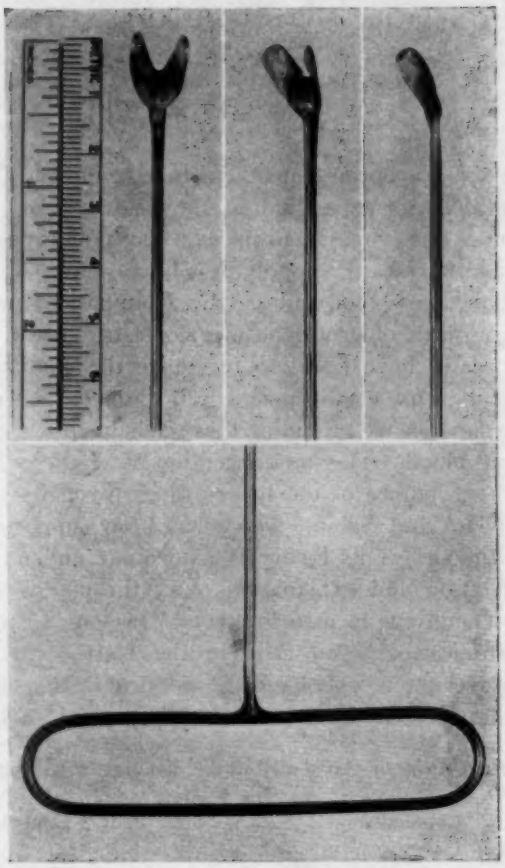
This instrument consists of a blade mounted on a spring steel shaft measuring 50 cm. in length and 0.2 cm. in diameter. At the opposite end the shaft is bent into a loop, which forms the handle. The blade consists of a V-shaped piece of German silver measuring 1.3 cm. in length and 1.5 cm. in width. The apex is united to the shaft by a rivet, followed by soldering. Union by either method alone is not satisfactory. The two arms of the V are bent forward so that they make an angle of about 45 degrees with each other and form between them a smaller V-shaped recess which has sharpened edges. The outer ends and edges are rounded and dull, so that this portion of the instrument will not impinge on the bony walls of the vertebral canal or incise the cord itself. The arms of the V-shaped blade make an angle of about 25 degrees with the axis of the shaft, though the apex formed by the cutting edges is on a line with the shaft.

The cord is removed in the following manner: After removal of the brain the anterior cervical and dorsal thoracic curves are straightened by placing a block beneath the thoracic portion of the spinal column and by elevating the occipital portion of the skull. The upper roots on one side of the divided spinal cord are grasped with a Mayo clamp, and slight tension is placed on the cord. The instrument is then pushed along one side of the cord within the subarachnoid space with its concave surface facing the convex lateral surface of the spinal cord. As the instrument passes down the vertebral canal, the motor and sensory roots and the dentate ligament are gathered into the V-shaped recess and are severed by its sharpened inner edges. When the blade has severed the roots down to the lumbar region, it is removed and the roots on the opposite side are divided in a similar manner. The lower end of the cord, or cauda equina, is transected after exposure of the vertebral canal by transecting one of the intervertebral disks at the appropriate level. The cord is then gently removed by pulling it upward through the foramen magnum.

With this procedure the spinal cord may be removed satisfactorily for routine examination with little or no gross or histologic evidence of trauma. The instrument has been used successfully in cadavers of all ages from 6 to 7 months upward. Smaller but similarly constructed blades might easily be substituted for use in smaller infants or animals. The instrument works poorly, however, in cases in which there is marked thoracic kyphosis, scoliosis or hypertrophic arthritis. In these cases and when an expanding lesion of the cord which would obliterate the spinal canal is suspected, i. e., a neoplasm, it is preferable to expose the cord in the usual manner by removing the posterior portion of the spinal column.

From the Division of Pathology, University of California Medical School.

1. Brines, O. A.; Webster, J. E., and Chesney, A.: Arch. Path. **22**:390, 1936.



Above: anterior, oblique and lateral views of the blade. Below: loop handle of the shaft.

General Reviews

MORPHOLOGIC, PHYSIOLOGIC, CHEMICAL AND BIOLOGIC DISTINCTION OF MEGALOBLASTS

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For many years European hematologists have utilized the recognition of genuine megaloblasts as an aid in the diagnosis of severe anemic conditions. This is due primarily to the excellent publications by Pappenheim, Naegeli and Ferrata. Secondly, it is because most of the European hematologists have used blood and marrow from patients with pernicious anemia as their source of prototype megaloblasts. Some hematologists in the United States have failed to recognize the genuine megaloblast as a pathologic type of erythropoietic cell and consequently have not appreciated the significance of finding these cells in either sternal marrow or the peripheral blood. This misconception of erythropoiesis is due to the widespread acceptance of the theory of erythropoiesis advanced by Doan, Cunningham and Sabin,[1] which has been supported in part by Isaacs [2] and Osgood.[3] This theory is inadequate and unsuited for the proper interpretation and explanation of erythropoiesis under normal and pathologic conditions in man for several reasons. First, it is based on Sabin's [4] studies of blood cells in the blastoderm of the chick embryo, which are not morphologically identical with genuine megaloblasts and are not present in normal chick marrow.[5] Secondly, Doan,

From the Department of Anatomy of the University of Buffalo School of Medicine.

1. Doan, C. A.; Cunningham, R. S., and Sabin, F. R.: Contrib. Embryol. **16**: 163, 1925.

2. Isaacs, R.: The Erythrocytes, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 1, p. 5. In a recent personal communication Isaacs indicated that he has changed his views regarding megaloblast. He now believes that when the megaloblast does not mature in the absence of hepatic principle, it undergoes marked changes in nuclear and cytoplasmic area as it lingers in the marrow. The end result is that a larger erythrocyte is produced. He also believes there are certain analogies to tumor cell formation in the body.

3. Osgood, E. E., and Ashworth, C. M.: Atlas of Hematology, San Francisco, J. W. Stacy, Inc., 1937.

4. Sabin, F. R.: Bull. Johns Hopkins Hosp. **32**:314, 1921.

5. Jones, O. P.: Unpublished data.

Cunningham and Sabin indiscriminately redefined the term "megaloblast" to include primitive erythroblasts of the embryonic yolk sac, pronormoblasts (proerythroblasts) of the fetal liver and normal marrow and promegaloblasts of the marrow of patients with pernicious anemia.[6] Maximow [7a] attempted to justify his own misuse of the term "megaloblast" [7b] as well as that of Doan, Cunningham and Sabin by claiming no reference was made to the cell's being related to the pathologic erythroblast of pernicious anemia or to "Ehrlich's megaloblast." This does not hold for Sabin and Miller,[8] since they are of the opinion that the youngest red cells in normal rabbit marrow are of the same type as the most immature erythroid forms seen in the blood in pernicious anemia. Thirdly, in view of the dominant position which the megaloblast has in the theory of erythropoiesis advanced by Doan, Cunningham and Sabin, it is disconcerting to find no record that they ever studied tissue known to possess genuine megaloblasts while they were formulating their theory from 1921 to 1925.[5] And finally, the so-called confirmation of the Doan, Cunningham and Sabin theory by Peabody,[9] Muller,[10] Sabin and co-workers [11] and Sabin and Miller [8] was based on a study of marrow in which genuine megaloblasts are known to be lacking.[5] It should be realized that this criticism is leveled toward only one part of their theory and does not take into consideration the arguments regarding the retention of embryonic potencies by endothelial cells in the adult [12] nor the presence or the absence of intersinusoidal capillaries in normal marrow.[13] Jordan and Johnson [14] have made certain remarks concerning the confirmation of the Doan, Cunningham and Sabin concept of erythropoiesis which bear repetition, viz.: "It seems desirable that a result of such magnitude, considering its wide application especially in

6. Jones, O. P.: (*a*) Anat. Rec. (supp. 3) **70**:42, 1938; (*b*) Proc. Soc. Exper. Biol. & Med. **38**:222, 1938.

7. Maximow, A.: (*a*) Bindegewebe und blutbildende Gewebe, in von Mollendorf, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, pt. 1, p. 232; (*b*) Folia haemat. **4**:612, 1907; Arch. f. mikr. Anat. **73**:444, 1909.

8. Sabin, F. R., and Miller, F. R.: Normal Bone Marrow, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 3, p. 1791.

9. Peabody, F. W.: Am. J. Path. **2**:487, 1926.

10. Muller, G. L.: J. Exper. Med. **43**:533, 1926; **45**:399, 1927; Am. J. Physiol. **82**:269, 1927.

11. Sabin, F. R.; Miller, F. R.; Smithburn, K. C.; Thomas, R. M., and Hummel, L. E.: J. Exper. Med. **64**:97, 1936.

12. Bloom, W.: The Embryogenesis of Mammalian Blood, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 2, p. 863.

13. Ringoen, A. R.: Anat. Rec. (supp. 4) **58**:84, 1934; (supp. 4) **61**:42, 1935. McDonald, J. G.: Am. J. Anat. **65**:291, 1939.

14. Jordan, H. E., and Johnson, E. P.: Am. J. Anat. **56**:71, 1935.

pathology, should be further tested by additional investigations. There are few similar instances where a concept of such wide and important bearings has been so completely accepted with so little effort at verification."

MORPHOLOGIC DISTINCTION OF MEGALOBLASTS

Evidence has been accumulating steadily in support of the contention that megaloblasts are found almost entirely, if not exclusively, in patients with macrocytic anemia of the type due to a deficiency of liver principle. Limarzi [15] found this to be so in a series of 2,215 or more sternal marrows, and Rohr,[16] in a series of more than 1,800. Hence, to the disciplined eye the presence of a megaloblastic marrow not only separates this type of anemia from other types of macrocytic anemia but means that in an uncomplicated case the patient can be treated efficaciously with potent liver extract or desiccated porcine stomach.[17]

It is not the purpose of this paper to discuss at length the terminology, the structure and the origin of megaloblasts, their relationship to primitive erythroblasts of the yolk sac or their fate after liver therapy, for these subjects are treated elsewhere.[18] However, it should be borne in mind that the genuine megaloblast is the predominant type of red cell developed in the marrow of patients with pernicious anemia during severe relapses. The youngest megaloblasts are usually derived from the myeloblasts and rarely directly from the reticuloendothelium.[18a] The end products of the megaloblastic series are reticulated and mature megalocytes, which are responsible for the increases in color index, corpuscular diameter and mean corpuscular volume in the peripheral blood of patients with pernicious anemia.[6a]

The consideration of the megaloblastic series as a pathologic type of erythropoiesis is based on the following morphologic evidence. The

15. Limarzi, L. R.: Personal communication to the author.

16. Rohr, K.: (*a*) Knochenmarksmorphologie des menschlichen Sternalpunktats, Berlin, Urban & Schwarzenberg, 1937; (*b*) Das menschliche Knochenmark, Leipzig, Georg Thieme, 1940.

17. (*a*) Koller, F.: Deutsches Arch. f. klin. Med. **184**:568, 1939. (*b*) Wilson, T. E.: M. J. Australia **29**:513, 1942. (*c*) Watson, C. J.: Illinois M. J. **82**:195, 1942.

18. (*a*) Jones, O. P.: Cytology of Pathologic Marrow Cells, with Special Reference to Bone Marrow Biopsies, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 3, p. 2045. (*b*) Rohr.[16] (*c*) Fieschi, A.: Semeiologia del midollo osseo: Studio di morfologia clinica, in Ferrata, A.: Biblioteca "Haematologica," Pavia, Tipografia gia cooperativa, 1938; Ergebn. d. inn. Med. u. Kinderh. **59**:382, 1940. (*d*) Schulten, H.: Die Sternalpunktion als diagnostische Methode, Leipzig, Georg Thieme, 1937. (*e*) Maximow.[7a] (*f*) Naegeli, O.: Wien. klin. Wchnschr. **48**:225, 1935. (*g*) Koller.[17a] (*h*) Bock, H. E., and Malamos, B.: Folia haemat. **62**:408, 1939. (*i*) Schartum-Hansen, H.: ibid. **58**:145, 1937. (*j*) Lambin, P., and de Weerdt, W.: Rev. belge sc. méd. **10**:282, 1938.

nuclear structure of the promegaloblast is quite different from that of any cell in normal marrow and especially that of the pronormoblast (proerythroblast).[19] It is composed of a chromatin network which is very finely stippled or divided into small thickened knots and masses. These are uniformly distributed throughout the nucleus and are separated by an equal or even greater amount of slightly acidophilic parachromatin. A tendency for the chromatin to clump is found only in the vicinity of the nucleoli, which are faint and not sharply outlined.[20] Even though the vast majority of promegaloblasts can be traced back to an origin from myeloblasts (lymphoidocytes),[21] they do have a nucleus which bears some resemblance to that found in histiocytes or reticulum of the hemopoietic tissue. This peculiar reconstruction of the nuclear pattern after cells have passed beyond the myeloblast (lymphoidocyte) stage may be the reason some authors have believed all megaloblasts in all cases are derived directly from the reticuloendothelium.[21f] In the later stages of megaloblastic development, when the cytoplasm is polychromatophilic or acidophilic, the chromatin has a tendency to fuse into larger discrete clumps, which may be connected to one another by fine threads of chromatin. These irregular chromatin masses are surrounded by an abundant acidophilic parachromatin, which gives the nucleus a dappled or speckled appearance. The general pattern in megaloblastic nuclei persists even though the cytoplasm is completely acidophilic. Remnants of this characteristic nuclear pattern remain discernible until shortly before pyknosis.[22] This type of nuclear pattern is never found in cells of the normoblastic series. The diameter of young megaloblasts may reach 40 microns, which is much greater than the usual 15 to 25 microns of pronormoblasts in normal marrow.[23] The nucleocytoplasmic ratio is disturbed by a marked increase in the amount of cytoplasm. The latter has a peculiar deep blue basophilia, which is not as homogeneous as the basophilia of pronormoblasts.[18a]

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19. (*a*) Jones,[18a] plates I, III and IV. (*b*) Wintrobe, M. M.: Clinical Hematology, Philadelphia, Lea & Febiger, 1942, plate 2, figs. 10 and 12. (*c*) Fallon, M.: Classification of the Anemias and Anemias in Infants and Children, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 3, p. 2175, plate 1. (*d*) Maximow, A., and Bloom, W.: A Textbook of Histology, ed. 4, Philadelphia, W. B. Saunders Company, 1942, p. 90, fig. 71.

20. Jones.[18a] Rohr.[16] Jones.[5]

21. (*a*) Jones, O. P.: Anat. Rec. (supp. 4) **58**:23, 1934; (*b*) (supp. 4) **61**:28, 1935; (*c*) The Origin and Structure of Erythroblasts with Special Reference to the Megaloblast, Thesis, University of Minnesota, 1935; (*d*) Proc. Soc. Exper. Biol. & Med. **34**:694, 1936. (*e*) Jones.[18a] (*f*) Tischendorf, W.: Deutsches Arch. f. klin. Med. **187**:556, 1941.

22. Jones.[18a] Jones.[5] Rohr.[16] Klima, R.: Sternalpunktion und Knochenmarksbild bei Blutkrankheiten, Berlin, Urban & Schwarzenberg, 1938.

23. Rohr.[16] Jones.[5]

Neither multipolar mitoses nor multinucleated giant megaloblasts are uncommon.[24] The chromosomes in promegaloblasts and basophilic megaloblasts tend to be finer and more slender than those of normoblasts.[25] However, even greater alterations of chromosomes and nuclear activities are found in the later stages of megaloblastic development, when the cells are more fully hemoglobinized. There is a tendency for degenerative mitoses to occur in acidophilic megaloblasts which are approaching pyknosis. The chromosomes are much shorter and stumpier than normal. In some instances pyknosis and fusion of chromosomes bring about an apparent modification of the number. Fieschi [18c] suggested that perhaps cells which are undergoing rapid proliferation pass beyond the stage in which pyknosis and denucleation normally take place, thereby accounting for the abnormal chromosomes. Or the abnormality may be related to the physicochemical state of the protoplasm, which is impossible to determine accurately. Fieschi [26] has also described a lagging behind of some chromosomes during mitosis, which gives rise to aberrant chromatin masses. The end result of such a mechanism has been seen in a case in which, following the reconstruction phase, there were two extranuclear chromosomes in the cytoplasm adjacent to an intact nuclear membrane. These extranuclear chromosomes were found in a basophilic megaloblast, and the phenomenon has not been observed in any other type of blood cell so far.[5]

Although hemoglobiniferous cells are for the most part devoid of any cytoplasmic granulation when routine hematologic stains are employed, there have been exhibited basophilic megaloblasts with atypical azurophilic granulation in a few cases of pernicious anemia.[27] A somewhat different type of granulation has been found in acidophilic megaloblasts after liver therapy.[6b] Recently Sergerdahl [28] reported that this peculiar vaculogranular degeneration of megaloblasts is seen quite regularly in cases of pernicious anemia of pregnancy. As to whether all of these cytoplasmic structures are the same is a subject for further investigation. The late stages of megaloblastic development usually show marked karyorrhexis or karyolysis or both. Karyolysis may lead to the formation of Cabot's ring bodies, which have been found even in hemo-

24. (*a*) Rohr.[16] (*b*) Fieschi, A.: Haematologica **17**:125, 1936. (*c*) Fieschi.[18c] (*d*) Limarzi, L. R.; Levinson, S. A., and Jones, R. M.: J. A. M. A. **118**:1004, 1942. (*e*) Limarzi, L. R.; Jones, R. M., and Levinson, S. A.: Anat. Rec. (supp. 3) **79**:43, 1941.

25. Rohr.[16] Fieschi.[18c] Schwarz (Am. J. Cancer **33**:120, 1938) was able to show that the diploid number of 48 chromosomes in man also holds for megaloblasts.

26. Fieschi.[18e] Fieschi.[24b]

27. (*a*) Segerdahl, E.: Ueber Sternalpunktionen, Uppsala, Appelbergs Boktryckeriaktiebolag, 1935. (*b*) Jones, O. P.: Folia haemat. **55**:195, 1936.

28. Sergerdahl, E.: Acta med. Scandinav. **108**:483, 1941.

globiniferous cells with an intact nucleus.[29] However, these views have been discredited in the recent work by Schleicher,[29a] who asserted that Cabot ring bodies are not nuclear remnants and are not identical with the nuclear membrane but are laboratory creations—the expression of cellular degeneration induced by hemolytic agents. Cabot ring bodies represent an expression of aggregated and denatured colloid protein.

The megaloblasts of pernicious anemia and the primitive erythroblasts of mammalian embryos have been differentiated from each other on the basis that the latter fail to enucleate rapidly and seldom undergo karyorrhexis.[30] Livadas [31] described the embryonic cells as larger, more hyperchromic and having more luster and gloss. The pathologic megaloblasts also differ from primitive erythroblasts in that they are formed extravascularly in the marrow, the liver and the spleen along with the abnormal neutrophils, whereas the primitive erythroblasts are formed intravascularly only in the embryonic yolk sac under physiologic conditions.[32] Moreover, recent experiments indicate that primitive erythroblasts—quite unlike megaloblasts—will respond to massive doses of iron alone.[32a]

The end products of the megaloblastic series are non-nucleated and larger and contain more hemoglobin per corpuscle than normal erythrocytes. Jaffé [33] described the megalocyte as "a real functional giant which is doomed to quick destruction, since it is an abnormal cell." Megalocytes also differ from normal erythrocytes in that they are more oval.[34] Most megalocytes pass through a stage in which they have reticulation; some, however, do not.[6]

PHYSIOLOGIC AND CHEMICAL DISTINCTION OF MEGALOBLASTS

Morphologically the marrow in pernicious anemia is characterized chiefly by very active proliferation of hemoglobiniferous cells foreign to normal marrow, which are in all phases of maturation. This is contrary

29. Isaacs.[2] Tsamboulas, N., and Malikiosis, X.: Deutsches Arch. f. klin. Med. **184**:183, 1939.

29a. Schleicher, E. M.: J. Lab. & Clin. Med. **27**:983, 1942.

30. Kirschbaum, A.: Blood Cell Formation in Mammalian Embryos from the First Appearance of Vascular Cellular Elements Through the Period of Hepatic Hemopoiesis, Thesis, University of Minnesota, 1936; Proc. Soc. Exper. Biol. & Med. **35**:542, 1937.

31. Livadas, K.: Folia haemat. **49**:365, 1933.

32. Maximow.[7a] Naegeli.[18f] Lividas.[31] Rohr.[16] Jones.[5]

32a. Jones, O. P.: Anat. Rec. **85**:321, 1943. These investigations were aided by a grant from the Committee on Scientific Research of the American Medical Association.

33. Jaffé, R. H.: J. A. M. A. **107**:124, 1936.

34. Schulten.[18d] Schartum-Hansen.[18i] Rohr.[16] Koller.[17a] Isaacs.[2] Schulten, H.: Folia haemat. **57**:189, 1937.

to Peabody's view that there is a proliferation and maturation arrest of the young hemoglobiniferous cells normally present in marrow.[35] Peabody's misconception regarding the erythropoiesis occurring in pernicious anemia during relapse is partly due to the fact that he had little or no faith in the use of dry smears or imprints for studies of marrow. When Peabody [35d] used sections alone, he sacrificed accurate identification of cells for knowledge of the general structure of marrow.[5] The value of dry smears for demonstrating nuclear structure has been reemphasized recently by Sundberg and Downey [36] and even by Cunningham's co-worker, Schwind.[37] The fact that Peabody did not accurately identify the cells of normal marrow or properly interpret the cells that develop in the marrow during relapse in pernicious anemia does not detract from his brilliant work of showing that marrow of the latter type returns to normal after the administration of liver.

Not only is there morphologic evidence to support the concept of the megaloblastic series representing a pathologic type of erythropoiesis but there is some which indicates that these cells are both chemically and physiologically abnormal. Studies of the metabolism of pigment (specifically the rate of excretion of coproporphyrin I) have clearly demonstrated that in pernicious anemia the marrow is abnormally active during relapse and less so during remission.[38] This is further evidence against Peabody's concept of an arrest of maturation during relapse in pernicious anemia. The megaloblastic cells which are responsible for this activity consume oxygen at a rate which is below the lower limit of the normal range for sternal marrow. In contrast to this, normoblastic (erythroblastic) marrow from patients with simple hypochromic anemia has a rate two or three times the normal.[39] Schretzenmayer and Brö-cheler [39] maintained that this supports Naegeli's contention that there is a definite difference between megaloblasts and normoblasts (erythroblasts). It is difficult to reconcile the difference in consumption of oxygen if substantia granulofilamentosa (reticulation) plays a role in

35. (*a*) Jones.[18a] (*b*) Jones, O. P.: Arch. Int. Med. **60**:1002, 1937. (*c*) Fieschi.[18c] (*d*) Peabody, F. W.: Am. J. Path. **3**:179, 1927.

36. Sundberg, D., and Downey, H.: Am. J. Anat. **70**:455, 1942.

37. Schwind, J. L.: Anat. Rec. (supp. 2) **79**:55, 1941. In a personal communication Schwind (1942) expressed the opinion that the misunderstanding of the nature of megaloblasts was due in part to the excessive enthusiasm about the possibilities of the supravital method and the use of this method for purposes to which it is not well adapted.

38. (*a*) Watson, C. J.: J. Clin. Investigation **16**:383, 1937; (*b*) The Pyrrol Pigments with Particular Reference to Normal and Pathologic Hemoglobin Metabolism, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 4, p. 2447. (*c*) Dobriner, K., and Rhoads, C. P.: J. Clin. Investigation **17**:95, 1938.

39. Schretzenmayer, A., and Bröcheler, H.: Klin. Wchnschr. **15**:998, 1936.

the respiration of cells, since both cell types possess an abundance of it in their early developmental stages. Bock and Malamos [18b] studied 17 specimens of sternal marrow taken from a patient with pernicious anemia at intervals throughout a three and a half month period. Their studies of the marrow's consumption of oxygen revealed a high value prior to treatment and a marked decrease four days later. On the sixth day following the start of therapy, when the reticulocytes were at their peak, the consumption of oxygen increased again. Although Bock and Malamos found it difficult to explain this secondary increase when the cells were becoming more mature, it can very likely be attributed to the high percentage of reticulocytes rather than to some unknown respiratory function in hemoglobin-laden erythroblasts, as they described it. The obvious discrepancy between the investigations of these two groups of workers indicates a necessity for further work along these lines. Damblé [40] reported that the oxygen consumption of the megalocytes of pernicious anemia as well as that of the erythrocytes of secondary hypochromic anemia with nonreactive marrow is less than normal, but that the oxygen consumption of the erythrocytes of secondary hypochromic anemia with a reactive marrow is almost twice normal. He expressed the belief that these differences are dependent on the erythropoietic function of the marrow. Henderson,[41] Richards and Strauss [42] and Isac, Matthes and Yamanaka [43] have all shown that the hemoglobin of megalocytes in the blood of patients with pernicious anemia has a smaller affinity for oxygen than the hemoglobin of normal human blood. Deutsch [44] found that the increase of respiration produced in the blood of pernicious anemia by stimulation of the respiratory supplement (Ringer solution extract of fresh rat liver) is far less than that of normal blood. He claimed this may be accounted for by an alteration of the structure of the cell surface on which the oxidation takes place.

The permeability of megalocytes to dextrose, malonamide and thiourea is increased, whereas glycerin permeates more slowly.[45] Bang and Ørskov [45] explained this by assuming that the structure or the function of the cell membrane has a peculiar quality. The megalocytes of pernicious anemia have abnormalities in chemical composition which are indicative of a deficient corpuscular structure.[46] These abnormalities

40. Damblé, K.: Ztschr. f. d. ges. exper. Med. **86**:595, 1933.

41. Henderson, L. J.: Blood: A Study in General Physiology, New Haven, Yale University Press, 1928.

42. Richards, D. W., and Strauss, M. L.: J. Clin. Investigation **4**:105, 1927.

43. Isac, C.; Matthes, K., and Yamanaka, T.: Arch. f. exper. Path. u. Pharmakol. **189**:615, 1938.

44. Deutsch, W.: Biochem. J. **28**:2002, 1934.

45. Bang, O., and Ørskov, S. L.: J. Clin. Investigation **16**:279, 1937.

46. Williams, H. H.; Erickson, B. N.; Bernstein, S.; Hummel, F. C., and Macy, I. G.: J. Biol. Chem. **118**:599, 1937.

are excessive amounts of cholesterol esters and a deficiency of phospholipid and free cholesterol. The cation and anion content, chiefly potassium and hemoglobin, respectively, are both increased. Williams and co-workers [46] claimed that "if one accepts the current view with regard to physiological activity, the lipid and mineral composition of the abnormal erythrocytes in pernicious anemia during relapse indicates that the cells are not only in a state of lowered function or activity, but degenerating and retrogressing." After treatment for pernicious anemia there is an elevation in total phospholipids, in cephalin and in sphingomyelin to values above normal, with the amount of lecithin definitely decreased.[47] Megalocytes have been found to have a high magnesium content in the majority of cases of pernicious anemia examined during relapse.[48] This is a property of newly formed cells which may also be found in anemias due to other causes. However, Bang and Ørskov [48] expressed the belief that this lends support to their view that the increased destruction in pernicious anemia is brought about through abnormal fragility and short lifetime of red cells in the marrow and the blood stream. Whipple [49] has maintained for many years that there is a scarcity of building material for the stroma or an abnormality of the cells within the marrow that control the output of framework for the red blood cells.

Seggel [50] recently showed that the red fluorescent erythrocytes are less numerous than normal and frequently lacking in the blood of patients with pernicious anemia during relapse. This phenomenon is accompanied by a low value for the protoporphyrin content of erythrocytes. In general, the amount of red fluorescence in aspirated marrow is no greater than that found in the circulating blood. However, in rare isolated instances rose fluorescence was found in the cytoplasm of leukocytes and orange fluorescence in the nuclei of very young hemoglobiniferous cells. This is a distinct contrast to the absence of fluorescence in nucleated red cells and the grayish white to grayish blue fluorescence of leukocytes in normal bone marrow.

BIOLOGIC DISTINCTION OF MEGALOBLASTS

There are at least two substances which will cause megaloblasts to exhibit entirely different biologic reactions. The first is anti-pernicious-anemia principle and the second is arsenic. The exact nature of what happens to the pathologic marrow of patients with pernicious anemia during the first twenty-four to forty-eight hours after the administration

47. Williams, H. H.; Erickson, B. N.; Bernstein, S. S., and Macy, I. G.,: Proc. Soc. Exper. Biol. & Med. **45**:151, 1940.

48. Bang, O., and Ørskov, S. L.: J. Clin. Investigation **18**:497, 1939.

49. Whipple, G. H.: Arch. Int. Med. **29**:711, 1922; J. A. M. A. **104**:791, 1935.

50. Seggel, K. A.: Ergebn. d. inn. Med. u. Kinderh. **58**:582, 1940.

of specific antianemic therapy is still a moot question. It has been demonstrated conclusively by Weiner and Kaznelson,[51] Peabody,[35d] Nordenson,[52] Koller,[17a] Rohr,[16] Scott [53] and others that the megaloblastic marrow of relapse is eventually transformed into a normal-appearing marrow. The unsettled question is: By what mechanism does this dramatic conversion or transformation take place? Some maintain that megaloblasts complete their maturation as megalocytes and are not transformed into cells of the definitive or normoblastic series.[54] The latter reappear and return to normal function by heteroplastic development from the reticulum or the myeloblasts or both and by homoplastic development from the quiescent remnants of the normoblastic series present in the marrow during relapse.[55] Some support for the view that megaloblasts are unable to differentiate into cells of the normoblastic series is found in the recent experiments on the placental transfer of anti-pernicious-anemia principle [56]; one must bear in mind, however, that megaloblasts appear under pathologic conditions and that primitive erythroblasts appear under the physiologic conditions of the embryo.[57] Others have expressed the belief that megaloblasts, which previously lacked sufficient anti-pernicious-anemia principle to mature, are completely transformed into cells of the normal or definitive series after the institution of therapy.[58] Dameshek and Valentine [59] suggested there may be reestablishment of erythropoiesis along normal lines together with direct modification of megaloblasts to normoblasts. Wilson [17b] found no reason why, following liver therapy, the pathologic megaloblasts cannot be transformed into normoblasts or cells resembling normoblasts, which

51. Weiner, W., and Kaznelson, P.: Folia haemat. **32**:233, 1926.

52. Nordenson, N. G.: Studies on Bone Marrow from Sternal Puncture, Stockholm, Börtzells, Esselte, 1935.

53. Scott, R. B.: Quart. J. Med. **8**:127, 1939.

54. (*a*) Fieschi.[18e] (*b*) Mustafa, K.: Ztschr. f. klin. Med. **136**:416, 1939. (*c*) Naegeli.[18f] (*d*) Rohr.[16] (*e*) Segerdahl.[27a] (*f*) Storti, E.: Haematologica **18**:1, 1937; Gazz. d. osp. **59**:807, 1938. (*g*) Tischendorf.[21f] (*h*) Jones.[18a] (*i*) Jones.[6b] (*j*) Jones, O. P.: Arch. Int. Med. **68**:476, 1941. (*k*) Israëls, M. C. G.: Lancet **2**:207, 1941.

55. Jones.[6b] Jones.[54j] Fieschi.[18e] Tischendorf.[21f] Israëls.[54k]

56. Jones.[5] Jones.[54j] Jones, O. P.: Anat. Rec. (supp. 3) **73**:29, 1939; (supp. 2) **76**:34, 1940; (supp. 3) **79**:35, 1941; (supp. 3) **82**:77, 1942.

57. Recently these investigations have been aided by a grant from the Committee on Scientific Research of the American Medical Association.

58. (*a*) Doan, C. A.: Medicine **10**:323, 1931; J. Lab. & Clin. Med. **17**:887, 1932; Bull. New York Acad. Med. **15**:668, 1939. (*b*) Henning, N.: Deutsche med. Wchnschr. **39**:1543, 1935. (*c*) Houghton, B. C., and Doan, C. A.: Am. J. Clin. Path. **11**:144, 1941. (*d*) Isaacs.[2] (*e*) Osgood.[3] (*f*) Lambin and de Weerdt.[18j] (*g*) Peabody.[35d] (*h*) Sabin and Miller.[8] (*i*) Schulten.[18d] (*j*) Koller.[17a] (*k*) Schartum-Hansen.[18l]

59. Dameshek, W., and Valentine, E. H.: Arch. Path. **23**:159, 1937.

in turn proceed to form reticulocytes. Koller [17a] suggested that young multinucleated megaloblastic cells may subdivide and heteroplastically form a quantity of normoblasts. Limarzi and co-workers [60] also expressed the belief that since normal bipolar mitoses and maturation do not explain the rapid conversion of a megaloblastic marrow to a normoblastic one, multipolar mitoses and multinucleated erythroid cells, which are frequently seen in these marrows, may play a role. In even more recent studies Limarzi [61] attacked this problem by treating patients for pernicious anemia first with a solution of potassium arsenite (Fowler's solution) and then with potent liver extract. His tentative conclusion is that the arsenic-sensitive megaloblasts are not transformed into normoblasts.

In general, only patients having macrocytic anemia and megaloblastic marrow respond to specific anti-pernicious-anemia therapy. However, there have been rare patients with macrocytic anemia and genuine megaloblastic marrow who have failed completely to respond. This lack of response is thought to be due to failure of the marrow to utilize the specific active principle, or the marrow may have become exhausted.[62] On the other hand, patients with hyperplastic normoblastic or macronormoblastic marrow do not as a rule respond to the administration of anti-pernicious-anemia principle, even though the marrow may show considerable erythroid immaturity. However, a favorable response has been reported in a few cases of macrocytic anemia accompanying cirrhosis of the liver.[63] Castle and Minot [64] obtained an irregular response in similar cases.

Recently, Segerdahl and Davidson and others [65] have reported that some women with normocytic hypochromic and microcytic hypochromic anemia of pregnancy and puerperium have megaloblastic marrow quite identical with that found in patients with addisonian pernicious anemia at equivalent stages. These women responded to specific anti-pernicious-

60. Limarzi, Levinson and Jones.[24d] Limarzi, Jones and Levinson.[24e]

61. Limarzi, L. R.: Proc. Central Soc. Clin. Research **15**:12, 1942; J. A. M. A. **121**:1245, 1943.

62. (*a*) Wilkinson, J. F., and Israëls, M. C. G.: Brit. M. J. **1**:139 and 194, 1935. (*b*) Israëls, M. C. G., and Wilkinson, J. F.: Quart. J. Med. **5**:69, 1936. (*c*) Davidson, L. S. P.: Edinburgh M. J. **46**:474, 1939. (*d*) Wauchope, G. M., and Leslie-Smith, M.: Lancet **2**:1518, 1938. (*e*) Mahler, A., and Greenberg, D.: J. A. M. A. **112**:1150, 1939. Watson.[38b]

63. Wintrobe,[19b] Wintrobe, M. M.: Arch. Int. Med. **57**:289, 1936. Goldhamer, S. M.: ibid. **53**:54, 1934. Watson.[17e]

64. Castle, W. B., and Minot, G. R.: Pathologic Physiology and Clinical Description of the Anemias, New York, Oxford University Press, 1936.

65. (*a*) Segerdahl, E.: Acta med. Scandinav. **108**:483, 1941. (*b*) Davidson, L. S. P.; Davis, L. J., and Innes, J.: Brit. M. J. **2**:31, 1942. (*c*) Lambin, P., and de Weerdt, W.: Sang **13**:929, 1939.

anemia therapy after a variable refractory period. Since patients with color indexes within or below the normal range responded to such therapy, the aforementioned observers [65] have suggested that "the institution of rational treatment is dependent upon sternal puncture." Such a condition is seemingly contradictory to the concept developed in this paper thus far. Fortunately, it has been my privilege to examine specimens of sternal marrow from similar patients with microcytic hypochromic anemia and megaloblastic marrow. This material will be described eventually in more detail by Wolff and Limarzi.[15] My interpretation is that the hypochromic anemia developed because of a cessation of activity on the part of the normoblastic series. The megaloblastic series had not developed sufficiently to produce enough acidophilic (orthochromic) megaloblasts to furnish megalocytes for distribution in the peripheral blood. The latter in sufficient numbers would have produced a macrocytic blood picture. Material of this type is excellent for demonstrating morphologic differences between the normoblastic and the megaloblastic series. The marrow of the aforementioned patients presumably occupies a position intermediate to the marrow described by Lambin and de Weerdt [65c] in patients with very early pernicious anemia, in which megaloblasts were lacking, and that of patients with fully developed megaloblastosis of the marrow and macrocytic anemia. The fact that it responded to liver therapy emphasizes the need for recognizing genuine megaloblasts in bone marrow in order to determine the correct line of therapy. It is noteworthy that the pronounced development of the pathologic neutrophilic series (macropolycytes) in the marrow was reflected in the peripheral blood even in the absence of macrocytic anemia. Wolff and Limarzi [15] have suggested that the presence of these atypical neutrophils in the blood might aid in separating this from other microcytic or normocytic types of hypochromic anemia.

Prior to the advent of liver therapy, arsenic was widely used in the treatment of pernicious anemia although no real benefit from its use could be proved.[66a] Evans [66b] considered that its use as a stimulant to hemopoiesis might be "whipping up" an already overactive marrow and thereby hastening its final collapse. But Naegeli [18f] (as late as 1935) still maintained that such therapy produced unquestionable remissions. It has been known for many years that arsenic acts as an irritant of marrow and as a protoplasmic poison. In studies on the thymus, lymph nodes, Peyer's patches, marrow, intestinal epithelium and testicular tissue of the mouse, Dustin [67] showed that nuclear chromatin

66. (*a*) Minot, G. R., and Lee, R. I.: Boston M. & S. J. **177**:761, 1917. (*b*) Evans, F. A.: Pernicious Anemia, Baltimore, Williams & Wilkins Company, 1926.

67. Dustin, A. P.: Strasbourg méd. **85**:12, 1927.

passes through certain phases during which it is hypersensitive to arsenic. This reaction (karyoclasia) takes place as the nucleus approaches a phase of condensation, either chromosomal or prepyknotic.

Tempka and Braun [68] observed the effect of arsenotherapy administered to a man with pernicious anemia for forty-five days. During this period he received daily 150 mg. of arsacetin (sodium acetylarsinilate) by mouth and 5 mg. of arsenic acid subcutaneously for the first twenty-four days. Before this treatment the erythrocyte count was 1,250,000, the hemoglobin content 37 per cent, the leukocyte count 5,100 and the color index 1.5. After arsenotherapy the erythrocyte count was 3,210,000, the hemoglobin content 72 per cent, the leukocyte count 5,900 and the color index 1.1. The sternal marrow after seventeen days of arsenotherapy showed that while the promegaloblasts were unchanged, the other megaloblastic forms were somewhat reduced while the pronormoblasts and normoblasts were increased. The pathologic giant stab neutrophils, which are the precursors of the neutrophils in the peripheral blood of patients with pernicious anemia, were increased. Similar findings were present in the marrow forty-five days after the beginning of this therapy. From this it can be seen that arsenotherapy produces a slight remission in the peripheral blood by stimulating the marrow but that it does not influence qualitatively the pathologic composition of the marrow. Rohr [16b] reported the effects of arsenic on the marrow of 2 patients with pernicious anemia and illustrated in colors (fig. 17) many of the changes in megaloblasts. One patient who received 1 to 7 mg. of arsenic acid daily for seven days gave a negative response with an actual decrease in the number of erythrocytes and in the percentage of hemoglobin. Bone marrow aspirated on the seventh day after the commencement of therapy showed marked changes in the megaloblasts. Their nuclei were often swollen and misshapen. Extreme karyorrhexis (karyoclasia) with many bizarre nuclear protuberances was the commonest finding. Arsenotherapy was discontinued and injections of potent liver extract begun. Examination of the marrow eleven days later showed that the pathologic erythropoiesis had disappeared completely and that uniform macronormoblastic regeneration was established. Rohr [16b] summarized the effects of arsenic on the marrow in pernicious anemia by stating that it brings about pathologic stimulation of megaloblasts with hastening of cytoplasmic ripening, nuclear destruction and nuclear extrusion. This sometimes produces megalocytic remission in the blood but never normalization of the pathologic marrow. In other types of anemia arsenic stimulates normal erythropoiesis. Koller [17a] estimated the length of time it takes for basophilic megaloblasts to mature to megalocytes by studying sternal marrow from a patient with

68. Tempka, T., and Braun, B.: Folia haemat. **48**:355, 1932.

pernicious anemia (case 42) who was treated with 10 daily doses of arsenic acid (2 to 10 mg.). The marrow was examined before therapy and six and ten days afterward. On the sixth day polychromatophilic megaloblasts were more than doubled in number. On the tenth day acidophilic (orthochromatic) megaloblasts were increased about eleven times. From these data he calculated that it takes ten to twelve days for megalocytes to mature, in contrast to the three to four days for normocytes, following liver therapy. Koller was not unmindful of the fact that arsenic medication is unphysiologic.

In a preliminary report, Limarzi and associates [69] substantiated Rohr's findings in the marrow of pernicious anemia following arsenotherapy. They also studied marrow from patients with sickle cell anemia, erythroblastosis, hypochromic anemia, cirrhosis of the liver, carcinoma of the stomach, polycythemia vera and normal controls following oral administration of a solution of potassium arsenite (Fowler's solution). The striking thing was the inability of the drug to produce as extensive or corresponding changes in the normoblastic cells, even though some marrows exhibited marked erythroid immaturity. In cases of spherocytic hemolytic jaundice and 1 case of refractory anemia with microcytosis the administration of arsenic produced karyorrhexis in polychromatic and acidophilic normoblasts. However, in all instances the pronormoblasts remained intact. Hence, these findings have been interpreted as additional evidence for the separation of the pathologic megaloblastic series from the normoblastic series of red cell regeneration.

COMMENT

When Doan, Cunningham and Sabin [1] were formulating their theory of erythropoiesis, they placed more emphasis on the physiologic than on the morphologic approach, primarily because Sabin was of the opinion that these problems had passed beyond the morphologic stage.[70] Sabin was impressed by Naegeli's classification of leukocytes because it was functional and could be used in the clinical study of blood.[71] Using the same line of reasoning for the red blood corpuscles, one finds it most peculiar that Sabin did not accept any of Naegeli's views [72] regarding erythropoiesis, for the recognition of a megaloblastic and a normoblastic series of red cell development certainly has a functional basis.

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69. (*a*) Limarzi.[61] (*b*) Limarzi, L. R., and Levinson, S. A.: Proc. Inst. Med. Chicago **14**:232, 1942. (*c*) It has been my privilege to examine many of Dr. Limarzi's preparations and verify his interpretation of the effect of arsenic on megaloblasts.

70. Sabin, F. R.: (*a*) Physiol. Rev. **2**:38, 1922; (*b*) **8**:191, 1928.

71. Sabin,[70a] p. 44.

72. Naegeli, O.: Blutkrankheiten and Blutdiagnostik, ed. 4, Berlin, Julius Springer, 1923.

From a purely physiologic point of view the youngest hemoglobiniferous cells under embryonic, normal adult and pathologic conditions have only one function to perform, which is to begin synthesizing hemoglobin. There is, however, some evidence to indicate that in some of these conditions hemoglobin may differ.[73] Moreover, from the aforementioned distinction of megaloblasts it should be realized that these cells and their products are functionally as well as structurally abnormal, so that they are not just "the same old red cell" under different conditions.

It is possible to distinguish morphologically between the early hemoglobiniferous cells under certain conditions. The differences in nuclear structure between the primitive erythroblasts of the embryo, the promegaloblasts of pernicious anemia and the pronormoblasts (proerythroblasts) of normal marrow may be due to differences in extracellular environment, in intracellular metabolism, in the processes of the synthesis of hemoglobin or in a combination of these. Although the differences in nuclear structure seem insignificant to those who are unaccustomed to making a detailed examination of the nucleus, they are constant and appear under entirely different conditions. These cells should not be grouped together under one heading according to the scheme of Doan, Cunningham and Sabin [1] or that of Davidson, Davis and Innes [74] for at least two reasons: First, it would be impossible to determine the factors governing these differences in nuclear structure under various conditions. Second, a valuable morphologic diagnostic criterion would be buried.

Regardless of the controversy over the origin of megaloblasts and the fate of these cells following liver therapy, the fact remains that there is no argument among the majority of European clinical pathologists concerning the importance of recognizing and the implications of finding true megaloblastosis of the marrow. Furthermore, complications such as cancer, syphilis, diabetes mellitus, nephritis and hyperthyroidism apparently do not disturb this characteristic pattern of marrow in untreated pernicious anemia.[75] The ability to recognize and separate megaloblastic marrow from other erythroblastic marrows, such as those encountered in the anemias accompanying hepatic cirrhosis, gastric carcinoma and various hemolytic processes, should be cultivated and developed among students of clinical pathology in this country. However, this refinement of morphologic hematology will be retarded if clinical pathologists accept the claim of Sabin and Miller [8] that the marrow from a 2 week old rabbit is "as instructive for the study of maturation as the blood of a patient with pernicious anemia for the red series, or with myelogenous leucemia for the white cells." This holds true only for the progressive changes

73. Brinkman, R., and Jonxis, J. H. P.: J. Physiol. **85**:117, 1935.

74. Davidson, L. S. P.; Davis, L. J., and Innes, J.: Quart. J. Med. **11**:19, 1942.

75. Limarzi, L. R.: Illinois M. J. **75**:38, 1939.

from a basophilic to an acidophilic cytoplasm and not for the nuclear structure, since the latter is entirely different in the two situations.[76] It cannot be emphasized too strongly that the marrow from a 2 week old rabbit should not be used as classroom material to teach the important differential diagnostic characteristics of developing red cells in pernicious anemia.

As mentioned previously, Sabin and Miller [8] expressed the opinion that the youngest red cells in normal rabbit marrow are of the same type as the most immature erythroid forms seen in the blood in pernicious anemia. They show, in their figure 5, of a dry fixed smear, a cell labeled *Mg,* which is supposed to represent a megaloblast in marrow from a normal 5 day old rabbit. The nuclear structure is definitely like that of cells which have been described by Pappenheim, Naegeli, Ferrata, Maximow, Michels, Fieschi, Rohr, Schulten, Scott, Dameshek and Jones as belonging to the definitive or normoblastic series. As a matter of fact, it is a poor selection of a cell type which is to represent the earliest stage of any erythroid series since the nuclear structure is entirely too pachychromatic. If such a cell is compared with primitive erythroblasts of the rabbit yolk sac and with megaloblasts from the marrow of pernicious anemia during severe relapse, it is obvious that there is no morphologic justification for Sabin and Miller designating it as a "megaloblast." I should mention that after a demonstration of marrows from patients with pernicious anemia and normal rabbits at the meetings of the anatomists at the University of Pittsburgh (1938), Sabin finally conceded that cells morphologically identical with megaloblasts are not present in normal rabbit marrow.

Megaloblastosis of the marrow produces an increase of the mean corpuscular diameter and of the volume of the red cells in the peripheral blood. This megalocytic anemia is macrocytic, but it does not follow that all macrocytic types of anemia are necessarily megalocytic.[77] Therefore, it would be unjustifiable to deduce the presence of megaloblasts in the hemopoietic organs or the qualitative identity of blood pictures from peripheral macrocytosis alone.[78] Isräels [79] mentioned that certain types of hemolytic anemia and those accompanying carcinoma of the stomach have been assumed to possess megaloblastic erythropoiesis just because the Price-Jones curve has shown an increase in the cell diameters. However, no megaloblasts were present in the marrow.

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76. Jones.[18a] Jones.[54j] Kirschbaum.[30]

77. (*a*) Davidson.[62e] (*b*) Israëls, M. C. G.: J. Path. & Bact. **49**:231, 1939. (*c*) Wilson.[17b] (*d*) Scott.[53] (*e*) Jones.[54j] (*f*) McGowan, J. P.: Edinburgh M. J. **49**:568, 1942.

78. Davidson.[62e] Israëls.[77b] Jones.[54j]

79. (*a*) Israëls.[77b] (*b*) Israëls, M. C. G.: J. Path. & Bact. **52**:361, 1941. (*c*) Israëls.[54k]

Many macrocytic types of anemia are not related to a deficiency of liver principle of the addisonian or the pernicious type. As a group the macrocytic types are very heterogeneous, with perhaps as many different causes.[80] The diversity of this group can be illustrated by a list of conditions in which at some time during their course macrocytosis occurs. They are: Hodgkin's disease, aplastic or hyporegenerative anemia, multiple myeloma, carcinoma of the stomach, sigmoid colon and rectum, leukemia, pellagra, myxedema, lead poisoning, congenital and tertiary syphilis, metastatic tumors, malignant malaria, acquired or secondary acute hemolytic anemia, hepatic and splenic disease, acute rheumatic fever, erythroblastosis foetalis and reticuloendotheliosis.[81]

There are five fundamental cellular causes for an increase in the mean corpuscular diameter of red cells. They may produce macrocytosis either independently or, in some cases, in combination with one another. From a practical point of view, clinical pathologists should be able to recognize the first three of these fundamental causes of macrocytosis and be aware of the existence of the fourth. The first two fundamental causes of macrocytosis differ from each other primarily in size relationships. The third and fourth show differences in the origin and the maturation of the cells involved, whereas the fifth differs from all of the rest not only in the mode of origin and of maturation of the cells but also in that it is limited to the prehepatic period of embryonic development.

The first cause of macrocytosis in the peripheral blood may be a marked outpouring of reticulocytes from normal marrow. The macrocytosis here is due to the fact that reticulocytes or immature erythrocytes are larger than nonreticulated mature ones.[82] In cases of posthemorrhagic anemia, if the stimulation of the marrow has been great, a slight increase in the mean corpuscular volume may be present during the period of maximal reticulocytosis.[83]

The second cellular mechanism by which macrocytosis may develop is the presence of macronormoblastic marrow. This differs from normal marrow in that it is hyperplastic, the normoblasts (erythroblasts) are larger and there are more of the early forms (pronormoblasts and basophilic normoblasts). In general, this type of marrow is the one found in the macrocytic types of anemia not related to a deficiency of

80. Fallon.[19e] Davidson.[62e] Wintrobe.[19b] Wilson.[17b]

81. (*a*) Fallon.[19e] (*b*) Davidson.[62e] (*c*) Israëls.[77b] (*d*) Watson, C. J.: Ann. Int. Med. **12**:1782, 1939. (*e*) Watson, C. J., and Clarke, W. O.: Bull. Univ. Minnesota Hosp. **12**:356, 1940. (*f*) Dameshek, W., and Schwartz, S. O.: Medicine **19**:231, 1940. (*g*) Wilson.[17b] (*h*) Limarzi.[15] (*i*) Reisner, E. H.: Arch. Int. Med. **71**:230, 1943.

82. Isaacs.[2] Wintrobe.[19b]

83. (*a*) Wintrobe.[19b] (*b*) Wintrobe, M. M.: J. Clin. Investigation **13**:669, 1934.

liver principle. The large pronormoblasts present in these hyperplastic marrows may be the cells which some authors have misinterpreted as megaloblasts. However, careful examination will show that the resemblance is only superficial.[84] Another stumbling block may be due to the presence of myeloblasts with a very basophilic cytoplasm.[5] Dameshek and Schwartz [81f] measured the diameter of these nucleated red cells in the marrow from a patient with acute hemolytic anemia and found the mean diameter to be 1.0 to 1.2 microns larger than normal. The nuclear structure of the cells in macronormoblastic marrow is essentially the same as that in normal marrow with the exception that the nuclei are slightly larger. Not only are the reticulocytes larger but the mature nonreticulated corpuscles are larger than normal and hence can be properly designated as macrocytes. Consequently, it is possible to have macrocytic anemia which is not correlated with an increase in reticulocytes.[84a]

One of the best sources of material in which to study the structure of macronormoblasts is a dry smear or imprint of fetal liver.[85] Contrary to Dameshek and Valentine [59] and others,[86] megaloblasts are not present there. Recently Wilson [17b] erroneously reported young primitive erythroblasts present in fetal livers. The only primitive erythroblasts present in fetal livers are the late or acidophilic forms, which have been derived from mesenchymal precursors in the embryonic yolk sac.[87]

It is generally agreed that any process which interferes with the production, absorption, utilization or storage of the anti-pernicious-anemia principle will result in the production of megaloblastic marrow. Theoretically, patients with hepatic cirrhosis might be expected to show such alteration of marrow since it has been postulated that the storage of the liver principle is impaired in this disease. On the contrary, it has been the repeated experience of many hematologists that in the presence of cirrhosis of the liver the marrow is macronormoblastic in type.[88] As a matter of record, Israëls and Wilkinson [89] placed special emphasis on the

84. Dameshek and Schwartz.[81f] Wilson.[17b] Jones.[5]

84a. Watson and Clarke.[81e] Reisner.[81i] Watson.[17c]

85. Jones.[18a] Jones.[21c] Kirschbaum.[30]

86. (*a*) Aron, M.: Arch. de morphol. gén. et expér., 1922, no. 10. (*b*) Zanaty, A. F.: Virchows Arch. f. path. Anat. **293**:794, 1934. (*c*) Gilmour, J. R.: J. Path. & Bact. **52**:25, 1941.

87. (*a*) Kirschbaum.[30] (*b*) Bloom.[12] (*c*) Bloom, W., and Bartelmez, G. W.: Am. J. Anat. **67**:21, 1940.

88. (*a*) Limarzi.[75] (*b*) v. d. Merwe, C. F.: Folia haemat. **55**:218, 1936. (*c*) Rohr.[16] (*d*) Scott.[58] (*e*) Dameshek and Schwartz.[81f] (*f*) Wilson.[17b] (*g*) Benhamou, E.: Presse méd. **47**:755, 1939. (*h*) Markoff, N.: Deutsches Arch. f. klin. Med. **183**:289, 1938. (*i*) Revol, L.: L'exploration de la moelle osseuse par ponction sternale, Paris, J. B. Ballière et fils, 1938. (*j*) Dreyfus, C.: Schweiz. med. Wchnschr. **71**:682, 1941. (*k*) Jones.[5] Israëls.[54k]

89. Israëls, M. C. G., and Wilkinson, J. F.: Quart. J. Med. **9**:163, 1940.

fact that they have yet to see a case of hepatic cirrhosis in which megaloblastic marrow accompanied macrocytosis in the peripheral blood. Likewise, the absence of dysplastic marrow in cases of gastric carcinoma has been used to separate the anemia in this disease from true pernicious anemia.[90] However, the coincidence of pernicious anemia and gastric carcinoma, or the later development of gastric carcinoma in a patient with pernicious anemia, complicates this problem.

Doan,[58a] Sabin [1] and others [91] have maintained that megaloblasts may become more and more prominent as the demand on the blood-forming tissues increases. If this were so, marrow from patients with hemolytic anemia should have an increased number of these cells, especially during the period of a crisis. Here again, practically all hematologists are agreed that the marrow is hyperplastic and normoblastic or macronormoblastic in cellular composition.[92] In a few instances the pronormoblasts are atypical and arise directly from the reticulum,[93] which is very likely why Tötterman [94] mistook them for megaloblasts.

Dameshek and Schwartz [81f] made it a point to emphasize that the macrocytic anemia accompanying hemolytic processes is not due to the presence of orthochromatic macrocytes such as those seen in pernicious anemia. They suggested that this type of anemia should be classified as pseudomacrocytic since the macrocytosis is due to the presence of a large number of polychromatophilic reticulocytes. This does not seem to be warranted since "macrocytic" is a general term designating a quantitative attribute of the blood with no regard for the qualitative nature of the cells involved.

A megalocytic hypochromic anemia of pancreatic disease was reported by Cheney.[95a] Postmortem examination of marrow in 2 of the 6 cases revealed it to be hyperplastic and normoblastic (erythroblastic). Consequently the mechanism responsible for the increased corpuscular diameter was not a megaloblastic marrow as implied by the title of the article. Similarly, the macrocytic anemia in patients with erythroblastosis foetalis is not due to megaloblastosis as reported by Reisner.[81l] The large cells in his figure 11 have a nuclear pattern quite characteristic for cells in early stages of normoblastic or macronormoblastic

90. Wilkinson, J. F.: Acta med. Scandinav. **80**:466, 1933. Limarzi.[75] Merwe.[88b] Israëls.[77b] Rohr.[16] Jones.[5]

91. Steele, B. F.: J. Exper. Med. **57**:881, 1933. Tocantins, L. M.: Medicine **17**:155, 1938. Osgood.[3] Isaacs.[2]

92. Davidson.[62c] Israëls.[77b] Limarzi.[75] Dameshek and Schwartz.[81f] Israëls.[79b] Davidson, Davis and Innes.[74] Scott.[53] Wilson.[17b] Henning.[58b] Rohr.[16] Fieschi.[18c] Israëls.[54k]

93. Jones.[21b] Jones.[18a]

94. Tötterman, G.: Acta med. Scandinav. **90**:527, 1936.

95. (*a*) Cheney, G.: Folia haemat. **56**:28, 1936. (*b*) Anderson, G. W.: Am. J. Obst. & Gynec. **42**:1, 1941.

development. Even though Reisner has rearranged and modified the nomenclature used by Dameshek and Valentine [59] and Jones,[18a] those cells are certainly not genuine megaloblasts. This is further substantiated by the fact that my examination of material used by Dr. George W. Anderson in his study of nucleated red cell counts of fetal blood and blood of newborn infants failed to reveal the presence of megaloblasts.[95b]

The third cellular mechanism which produces macrocytosis in the peripheral blood is the megaloblastic marrow. Certain it is, however, that precocious hemoglobinization of erythroblasts in hyperplastic marrow should not be used as a criterion for the identification of megaloblasts.[96] This is admirably illustrated in a study of hemolytic anemia by Israëls,[97] in which he also emphasized that fine nuclear distinctions are lost in histologic sections. Furthermore, if Gilmour and Turnbull [96] were correct about megaloblasts being normal cells which have undergone precocious hemoglobinization, the youngest red cells in hyperplastic erythroblastic marrow should show the same sensitivity to arsenic as megaloblasts in pernicious anemia during relapse. This was not the case in the recent studies reported by Limarzi.[61] In all instances the pronormoblasts of such marrow were resistant to arsenic.

Although megaloblastic marrow is most frequently found in patients with anemia due to deficiency of liver principle, it must be admitted that it has been reported in totally unrelated conditions by authors who apparently were acquainted with the nuclear structure of genuine megaloblasts. The conditions in which these megaloblasts have been reported are: atypical myelosis with intense erythroblastosis; erythropoietic regeneration following irradiation of a cancer; localized erythropoietic hyperplasia in roentgen aleukia [16a]; erythroleukemia [18j]; monocytic leukemia [79b]; generalized tuberculosis [88c]; severe sepsis [68]; agranulocytosis [98]; leishmaniasis,[99] and carcinoma of the esophagus.[17b] Dameshek and Valentine [59] suggested that this sporadic occurrence of megaloblasts may be due to an unrecognized deficiency of liver principle. It is evident that megaloblasts alone are not pathognomonic for pernicious and related types of anemia, assuming that in the cases just mentioned there was no difference in the criteria for cellular identification.

It may be established eventually that the presence of genuine megaloblasts together with the giant stab form neutrophils, which are a part of the pathologic neutrophilic series,[100] is pathognomonic for this type of

96. Gilmour.[86e] Turnbull, H. M., in Vaughan, J. M.: The Anemias, London, Oxford University Press, 1934.

97. Israëls, M. C. G.: J. Path. & Bact. **52**:361, 1941.

98. Watson.[17e] Jones.[18a] Dameshek, W., and Ingall, M.: Am. J. M. Sc. **181**:502, 1931.

99. Kassirsky, I. A.: Folia haemat. **51**:352, 1934.

100. Jones.[21d] Jones.[35b] Jones.[18a]

macrocytic anemia. The presence of a pathologic neutrophilic series in the marrow of pernicious anemia is further indirect evidence to support my contention that the megaloblastic series is pathologic and represents neither a return to embryonic conditions nor an arrest of maturation in cells normally present in the marrow of adults.[101] Nordenson [52] and Henning [58b] claimed that abnormal neutrophils alone are pathognomonic of pernicious anemia. Wilson [17b] encountered giant stab form neutrophils only in pernicious anemia. In my material they have been found in marrow from patients with pernicious anemia, achrestic anemia, tropical macrocytic anemia, tropical sprue and pernicious anemia of pregnancy.[102] It is likely many American hematologists have not paid sufficient attention to alterations in the neutrophilic series and especially to the panmyelopathy which exists in the marrow of pernicious anemia during relapse.[100]

In considering this third mechanism which may produce a macrocytic blood picture, it should be realized that the megaloblastic series produces reticulated megalocytes as well as nonreticulated ones.[6b] The origin of poikilocytes will not be discussed here, since it has recently been considered elsewhere.[103]

The fourth cellular mechanism which may produce a macrocytic blood picture is an extremely rare condition, the occurrence of which has not been accepted generally nor has a sufficient number of cases been encountered to permit extensive study. Downey [104] and his students recognized a morbid condition that is characterized hematologically and histologically by irreversible hyperplasia of the reticulum with concomitant cellular differentiation of the liberated cells toward the hemoglobiniferous series and their circulation in the blood. This specific condition is one of the variants of leukemic reticuloendotheliosis.[104] In this disease the youngest nucleated red cells exhibit a nuclear structure which resembles that of the undifferentiated reticulum cells seen in dry smear preparations of hemopoietic organs. This reticular characteristic of the nucleus persists even though the cells are quite acidophilic. The final stage of this type of erythropoiesis is not unlike that of other erythroblasts in that the nucleus becomes pyknotic. This leukemic type

101. Jones.[100] Wilson.[17b] Rohr.[16] Fieschi.[18e]

102. Some of these marrows were furnished by the following men: Dr. L. R. Limarzi (pernicious anemia and pernicious anemia of pregnancy), the late Dr. I. J. Pass and Dr. C. J. Watson (achrestic anemia), Dr. W. M. James, Panama, Republic of Panama (tropical macrocytic anemia), Dr. R. Rodríguez-Molina, Puerto Rico (tropical sprue).

103. Tischendorf.[21f] Schultz, W., and Buding, A.: Deutsche med. Wchnschr. **66**:492, 1940.

104. Downey, H.: Monocytic Leucemia and Leucemic Reticulo-Endotheliosis, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 2, p. 1321, case 7.

of erythropoiesis differs from the reticular origin of megaloblasts and pronormoblasts [93] in that the reticular nuclear pattern is not limited to a single stage but may be found in the basophilic, polychromatophilic and acidophilic (orthochromatic) stages. These cells have skipped the myeloblast stage during their development from the reticulum.[104] This leukemic type of erythropoiesis also differs from the reticular origin of megaloblasts and pronormoblasts of other types of anemia in that it is an irreversible cellular change which has not been influenced by any known therapy.

In a case of my collection the non-nucleated forms were definitely macrocytic.[5] In the case reported by Limarzi and associates [105] the abnormal cells resembled megaloblasts more closely than they did cells of any other type with the possible exception that they might have been classed as a veritable erythroblastoma. The multinucleated giant erythroblasts formed non-nucleated corpuscles as much as 21 by 26 microns in diameter. To those accustomed to making a critical analysis of nuclear structure these leukemic cells are not genuine megaloblasts nor are they macronormoblasts. It is felt that the red cells in the case of "acute megaloblastic leukemia" described by Penati [106] really belong to this group, which may explain why it was so difficult to classify the cells.

The fifth cellular mechanism which aids in the production of a macrocytic blood picture is the development of the primitive erythroblastic series in the yolk sac of mammalian and avian embryos during the prehepatic period. These cells arise directly from mesenchymal elements of the yolk sac and are not formed in the fetal liver and spleen, nor in the adult bone marrow.[107] The megaloblasts of pernicious anemia are similar to but not identical with the primitive erythroblasts of the embryo.[76] The large non-nucleated products of this series circulate in the peripheral blood up to the time of birth in animals having a short period of gestation.[80] On the other hand, these cells disappear from the blood of human and pig fetuses a considerable time before birth.[80]

With these various morphologic mechanisms in mind it is possible to analyze qualitatively various blood pictures which have been grouped under one heading merely because they possess one quantitative attribute, namely, macrocytosis. It is not the purpose of this review to carry the analysis to completion but only to indicate a possible trend for future qualitative studies of macrocytic blood pictures.

The macrocytic blood picture in the late stages of human and pig fetuses, studied by Wintrobe and Shumacker,[108] is not produced by

105. Limarzi, Levinson and Jones.[24d] Limarzi, Jones and Levinson.[24e] Dr. Limarzi sent me duplicate bone marrow preparations from this case.

106. Penati, F.: Minerva med. **2**:401, 1937.

107. Kirschbaum.[80] Bloom and Bartelmez.[87e] Bloom.[12]

108. Wintrobe, M. M., and Schumacker, H. B.: J. Clin. Investigation **14**:837, 1935.

megalocytosis similar to that seen in persons with pernicious anemia, since megaloblasts are not present in the fetal liver and spleen.[76] If this blood picture is likened to that of any type of macrocytic anemia, it should be that seen in cases of hepatic cirrhosis in which there is a macronormoblastic marrow.[88] However, when it is considered that the fetal macronormoblasts are formed in the liver, whereas those in the cirrhotic patient are formed in the marrow, this similarity is not so simple.

The blood of the newborn rat has been used in the study of the effect of antianemic substances administered during gestation just because there is a quantitative similarity to blood from patients with pernicious anemia.[109] When the cellular mechanisms responsible for these two macrocytic blood pictures are studied, it is found that the two bloods are qualitatively quite different. For example, the macrocytic blood picture in pernicious anemia during severe relapse is produced by the products of the pathologic megaloblastic series, namely, the megalocytes and a few reticulated megalocytes. On the other hand, when the blood of the newborn rat is analyzed qualitatively, it is found that three different mechanisms are responsible for the final picture of macrocytosis.[54j] First, there are 90 per cent or more reticulocytes. The larger ones can be traced back to an origin from macronormoblasts in the liver, and the others have come from normoblasts in the marrow. The newborn rat also has a few cells circulating in its blood which are the end products of those originally formed intravascularly in the yolk sac during the prehepatic period.

When the increase in mean corpuscular volume [83] that occurs during liver therapy in cases of pernicious anemia is analyzed, it is found to be due to two different mechanisms.[6b] At the peak of a reticulocyte response there is an outpouring of immature red corpuscles, but this response is qualitatively different from other reticulocyte responses.[6b] It is composed of normal reticulocytes, reticulated megalocytes and mature megalocytes. The latter really represent the result of the purging of the marrow of its pathologic elements.[110] Mustafa [54b] measured cell diameters before and after liver therapy. At the height of the reticulocyte crisis he obtained a double peak curve, which has been interpreted to indicate an outpouring of megalocytes. Evidence to support my contention that the reticulocyte response in pernicious anemia is different from others [6b] is found in the studies by Cotti and others [111] on the uric acid output during this period.

In studying macrocytic blood pictures in man under various pathologic conditions, considerable attention should be directed to the qualita-

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109. Jones,[54j] pp. 481-483.

110. Jones.[56b] Tischendorf.[21f] Mustafa.[54b] Stasney, J., and Pizzolato, P.: Proc. Soc. Exper. Biol. & Med. **51**:335, 1942.

111. Cotti, L., and Balestrieri, F.: Gior. di clin. med. **20**:628, 1939. Stasney, J., and Pizzolato, P.: Proc. Soc. Exper. Biol. & Med. **51**:338, 1942.

tive analysis of the marrow cells as seen in dry smears. It should be realized that although there are four cellular mechanisms, each of which may produce a macrocytic blood picture, normoblastic erythropoiesis is always present to a variable extent. For example, even though the dominant type of erythropoiesis in the marrow in pernicious anemia is of the megaloblastic type, normoblasts are present. The latter may be dormant or slightly active. Lambin and de Weerdt [18j] and Schartum-Hansen [18l] postulated an intermediate form of erythroblast in pernicious anemia. That has not been necessary in my experience nor in that of Israëls.[77b] In hepatic cirrhosis, macronormoblasts dominate the picture, but there is a fairly good percentage of active normoblasts. The latter are also quite active in the hemolytic anemias. In leukemic reticuloendotheliosis [105] the giant bizarre multinucleated erythroblasts were the dominant feature, but the normoblastic series was proliferating quite actively.

From the foregoing review it should be evident that the megaloblast-normoblast problem is not merely a quibbling over the minutiae of terminology. To be sure, terminology has been confused. But to dismiss this whole problem of erythropoiesis by saying that it is entirely one of terminology is to admit a lack of knowledge concerning fundamental problems of erythropoiesis. The whole problem centers around the ability to recognize and appreciate differences in nuclear structure, the ability to separate pathologic from normal cells and the ability to separate normal or hyperplastic marrow patterns from dysplastic ones. These distinctions can be accomplished if good, fresh and well stained dry smears are studied. This technic brings out the delicate nuclear structure necessary if one is to discriminate between normal and pathologic erythropoietic cells. This cannot be done with the supravital technic. As Schwind [37] has pointed out, the latter is suited for a study of the cytoplasm, and there are no striking differences in the cytoplasm of the megaloblastic and normoblastic series, as there are in the nuclei.

Medical students should not be taught that the dominant type of erythropoietic cell seen in the marrow of pernicious anemia during relapse is present in normal marrow or that it has been readily produced experimentally (with one possible exception [112]), or that it increases with increasing demands on the blood-forming organs.

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112. Wills, L., and Stewart, A.: Brit. J. Exper. Path. **16**:444, 1935.

Forensic Medicine

DIFFERENTIATION OF FETAL AND ADULT HUMAN HEMOGLOBIN

ITS MEDICOLEGAL IMPORTANCE, ESPECIALLY IN CONNECTION WITH THE ALKALI TEST FOR CARBON MONOXIDE IN BLOOD

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That the hemoglobin of most animals is more resistant than human hemoglobin to the denaturizing action of alkalis was discovered in 1866 by Koerber.[1] His observations were confirmed by Krueger [2] and by Ziemke,[3] the latter making use of this discovery to differentiate human from animal blood. Bischoff [4] later found that hemoglobin from the human fetus resembled animal hemoglobin in that it was also strongly resistant to the denaturizing action of alkali and that it was unlike human adult hemoglobin, which is rapidly changed into alkaline hematin on the addition of alkali. Koerber [1] had already observed that human placental blood was more alkali resistant than adult blood.

Various methods may be employed to demonstrate the difference between fetal and adult hemoglobin in resistance to the action of alkali. The simplest method, that of Krueger,[2] can be carried out with a hand spectroscope. This method was used by Bischoff [4] and Trought.[5] More exact results may be obtained by the more complicated spectrophotometric method mentioned by Haurowitz [6] or by the photoelectric method used by Brinkman and his co-workers.[7] Recently,

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1. Koerber, E.: Ueber Differenzen des Blutfarbstoffs, Inaug. Dissert., Dorpat, 1866; cited by Ziemke.[3]

2. Krueger, F.: Ztschr. f. Biol. **24**:314, 1888; Ztschr. f. vergl. Physiol. **2**: 254, 1925.

3. Ziemke, E.: Vrtljschr. f. gerichtl. Med. **22**:77, 1901.

4. Bischoff, H.: Ztschr. f. d. ges. exper. Med. **48**:472, 1926. Bischoff, F., and Schulte, H.: Jahrb. f. Kinderh. **112**:56, 1926.

5. Trought, H.: Arch. Dis. Childhood **7**:259, 1926.

6. Haurowitz, F.: Ztschr. f. physiol. Chem. **183**:78, 1929; Ztschr. f. physiol. Med. **186**:141, 1930.

7. Brinkman, R.; Wildschut, A., and Witterman, A.: J. Physiol. **80**:377, 1934. Brinkman, R., and Jonxis, I. H. P.: ibid. **85**:117, 1935.

Darrow, Nowakowsky and Austin [8] prepared specific rabbit antiserums which react with and differentiate between human adult and fetal hemoglobin.

Fetal and adult hemoglobin differ in reaction to alkali because of the presence in fetal blood of two kinds of hemoglobin, one resistant and the other nonresistant to alkali. Haurowitz estimated that the blood of the newborn infant contains 80 per cent of resistant and 20 per cent of nonresistant hemoglobin. The amount of resistant hemoglobin diminishes gradually after birth; Brinkman and his co-workers found that it had disappeared entirely from the blood of infants 6 to 7 months of age. Trought stated that the resistant form of hemoglobin may disappear as early as four and a half months after birth, and Bischoff observed that it disappeared more rapidly from the blood of a full term baby than from that of a premature one. After the disappearance of the resistant form the hemoglobin of an infant cannot be differentiated from that of an adult.

The Krueger method for determining the resistance of hemoglobin to alkali is carried out in the following way:

> One cubic centimeter of a 1 per cent solution of sodium hydroxide is added to 5 cc. of an approximately 1 per cent solution of blood in a test tube. The untreated dilute blood solution is pinkish red, and when it is examined through the spectroscope, the two absorption bands of the oxyhemoglobin spectrum between D and E are plainly visible. The alkali which is added denatures the hemoglobin, changing its red color into the brown color of alkaline hematin and causing a disappearance of the two bands of the oxyhemoglobin spectrum. The denaturation time for the hemoglobin of adults or of infants 6 months of age or older is about one minute; the maximum, five minutes. The denaturation time for the hemoglobin of a fetus or a newborn infant is never less than one hour and more often two to three hours or longer. In many fetal bloods we have observed the absence of denaturation by alkali after twenty-four hours. The hemoglobin of infants from a few weeks to 3 months of age also exhibits a longer denaturation time than that of adults, the process requiring from one to two hours. The denaturation of adult hemoglobin into alkaline hematin is delayed slightly if a more concentrated solution of blood is used, but not sufficiently to confuse the results. The striking difference in resistance to the action of alkali between adult and fetal hemoglobin is evident even if more concentrated blood solutions are used for testing.
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> The alkaline hematin which is formed slowly from fetal hemoglobin is similar to that which is formed rapidly from adult hemoglobin in that it is transformed rapidly into hemochromogen on the addition of a few drops of dilute ammonium sulfide solution.

The difference between human adult and fetal hemoglobin in resistance to a dilute alkaline solution has considerable medicolegal importance, as this test may be used to differentiate between adult and fetal human blood stains. The alkali-resistant property of fetal hemoglobin inter-

8. Darrow, R.; Nowakowsky, S., and Austin, W.: Arch. Path. **30**:873, 1941.

feres with the commonly used test for the detection of carbon monoxide in blood, which is based on the resistance of carboxyhemoglobin to the action of alkali, in contrast to oxyhemoglobin. Obviously, the use of this test on the blood of a newborn or a very young infant might lead to a false positive result.

INTERFERENCE WITH TESTS FOR DETECTION OF CARBON MONOXIDE IN BLOOD

The Hoppe-Seyler [9] or alkali test of the blood is commonly employed in cases of carbon monoxide poisoning. The test will give a positive result if the blood is more than 15 per cent saturated with carbon monoxide. Thus, the addition of a few drops of a 10 per cent solution of sodium hydroxide to a dilute solution of normal blood immediately changes the latter's red color to the brown color of alkaline hematin. Blood containing the resistant carboxyhemoglobin in concentrations above 15 per cent when treated the same way retains its cherry red color for a considerable time. As already shown, similar results are obtained with normal blood from a human fetus or a newborn human infant having no trace of carbon monoxide in it.

Confusing results were obtained by the use of the sodium hydroxide test in an interesting case of carbon monoxide poisoning in which an autopsy was made in the Office of the Chief Medical Examiner (no. 3607-40).

A 37 year old white woman who was nine months pregnant committed suicide by inhaling illuminating gas. She was found dead in the gas-filled kitchen of her home. The body presented the typical cherry red postmortem lividity which one encounters when there is a high saturation of the blood with carbon monoxide, and the maternal blood and organs all showed the same bright red color. The uterus contained a well developed full term fetus, whose lividity was of a deep bluish color and whose blood and organs were dark colored, in contrast to the cherry red color in the mother. The fetal lungs were dark blue, firm, airless and sank in water.

Qualitative tests for carboxyhemoglobin made on the maternal blood were strongly positive, and there was no reduction in the carboxyhemoglobin spectrum after the addition of ammonium sulfide. All the chemical tests, tannic acid, sodium hydroxide, formaldehyde solution, acetic acid and ammonium sulfide, were positive for carbon monoxide. The saturation of the maternal blood with carbon monoxide was 65 per cent as determined by the Van Slyke method.

The alkali test on the fetal blood was also strongly positive, but the absence of carboxyhemoglobin in the fetal blood was indicated by the gross autopsy findings and also by all other tests for carbon monoxide, which were negative. The Hoppe-Seyler alkali test, which was the first used in this case and which is the one most commonly used in cases of suspected carbon monoxide poisoning, gave a false positive result. The fetus did not die of carbon monoxide asphyxia, as did the mother, but from simple asphyxia resulting from a lack of oxygen, which

9. Hoppe-Seyler, F.: Virchows Arch. f. path. Anat. **13**:104, 1858.

is normally supplied by the maternal blood through the placenta. This case offers additional evidence that in a woman with child there is no intermingling of the maternal and fetal circulations.

Maresch [10] reported a somewhat similar case, in which a pregnant young woman was acutely asphyxiated by carbon monoxide after she had inhaled illuminating gas in an attempt at suicide. She was found unconscious but was resuscitated. Twelve days later she delivered a full term infant, which lived nine days and which during life presented signs suggesting a cerebral injury sustained at birth. At necropsy, in addition to pneumonia, extensive softening of the basal ganglions was found. The lesion resembled the bilateral symmetric softening of the globus pallidus that occurs in most cases of delayed death from acute carbon monoxide poisoning and led Maresch to conclude that carbon monoxide had passed through the placenta into the fetal circulation. This conclusion, however, was not altogether warranted in the case, since chemical studies of the infant's blood obviously could not be carried out before birth, at the time that the mother was asphyxiated, and since the lesion may well have resulted from simple anoxia of the fetal brain tissues and not from any specific action of carbon monoxide. Bilateral symmetric softening of the globus pallidus may result from causes other than acute carbon monoxide poisoning. There are 2 cases recorded in the literature [11] in which the lesion developed after acute barbiturate poisoning, and a third instance of barbiturate poisoning with a similar lesion of the brain was recently observed by one of us (M. H.) but not reported. The case which we have described indicates that carbon monoxide does not pass through the placental circulation into the fetus.

The resistance of fetal hemoglobin to the denaturizing action of alkali was also observed in a case of sudden death from toxemia of pregnancy in a woman with a nine months' fetus in utero (no. 4744-42). The maternal hemoglobin was immediately changed into alkaline hematin after the addition of 1 per cent sodium hydroxide; the fetal blood was not denaturized even many hours after the addition of the alkali.

The alkali test should not be used for the detection of carbon monoxide in the blood of newborn or very young infants, as such blood will always give a false positive result because of the strong alkali-resistant quality of its hemoglobin.

Many observations which we have made indicate that the hemoglobin of human fetuses and newborn infants is even more resistant to the denaturizing action of alkali than is the carboxyhemoglobin found in

10. Maresch, R.: Wien. med. Wchnschr. **79**:454, 1929.

11. Gonzales, T. A.; Vance, M., and Helpern, M.: Legal Medicine and Toxicology, New York, D. Appleton-Century Company, Inc., 1940, p. 558. DeGroat A.: Arch. Path. **29**:271, 1940.

the blood of adults in acute carbon monoxide poisoning. In the case first described, the denaturation time of the fetal blood after treatment with 1 per cent sodium hydroxide solution was longer than that of the maternal blood, which was 65 per cent saturated with carbon monoxide.

In cases in which infanticide is suspected and in which the body of the infant has been partially incinerated, a false positive alkali test for carbon monoxide in the blood might lead to the erroneous conclusion that the infant had been burned alive. Such a case, in which the cause of death of a liveborn infant could not be determined and in which incineration occurred after death, has come to our attention.

All the other tests for carbon monoxide when used on fetal and infant blood are satisfactory and reliable. Fetal oxyhemoglobin is reduced to hemoglobin as rapidly as adult oxyhemoglobin after treatment with ammonium sulfide. Tannic acid, acetic acid and ammonium sulfide and formaldehyde solution gave reactions with normal fetal hemoglobin similar to those obtained with adult hemoglobin. In these tests grayish brown discolorations or precipitates are produced in contrast to the red color or red-colored precipitates obtained when these tests are carried out on blood containing carboxyhemoglobin. The difference in the resistance of fetal hemoglobin and adult hemoglobin to acids is insignificant and does not interfere with any of the tests for carboxyhemoglobin in which acids are used. Bischoff using twenty-five hundredth normal acetic acid found that a 1 per cent solution of human fetal hemoglobin is changed into acid hematin in twenty-three minutes, compared with adult hemoglobin, which is changed in thirteen minutes. With stronger acids, such as are used in testing for carboxyhemoglobin, there is no appreciable difference between adult and fetal hemoglobin in time taken for the formation of acid hematin.

DIFFERENTIATION OF HUMAN ADULT AND FETAL BLOOD AND BLOOD STAINS

The differentiation between fetal and adult blood by means of the alkali test can be carried out also on samples of blood taken during life, as well as at the autopsy table. Advanced decomposition and hemolysis of the blood do not interfere with this test. It was carried out successfully on samples of blood removed from cadavers and kept for several weeks in test tubes at room temperature. If hemoglobin is present in the decomposed blood samples, the difference in the denaturation time between fetal and adult hemoglobin is easily observed. Hemoglobin of human fetuses and newborn infants retains its resistance to alkali even when decomposed. The results of the denaturation test with alkali were in no way influenced by the cause of death.

Stains of blood from a fetus, a newborn infant and an adult were prepared on glass, paper, steel and other materials. These stains were preserved for periods of days, weeks and months at room temperature. It was observed that if a stain is only a few days old, it is not difficult to prepare an aqueous or a saline extract and that it is also possible to obtain extracts from stains which are several weeks old. The extracts from fresh blood stains can be diluted and treated with sodium hydroxide the same as fresh blood, with similar results.

Difficulty is encountered in testing blood stains which are more than several weeks old. The older stains usually have lost their red color and are brown as the result of the formation of methemoglobin during the process of drying. Extracts of these old stains are brown in color, and when examined with the spectroscope they reveal the band of methemoglobin in the red part of the spectrum. There is, however, some oxyhemoglobin which has remained unchanged and which is recognizable by its two characteristic bands in the green portion of the spectrum. If the stain is derived from fetal blood, the hemoglobin retains its strong resistance to alkali even after a long period of drying. In such cases the denaturation time can be observed only with the spectroscope, because the color of the extract of an old stain is already brown.

With extracts of old stains, the oxyhemoglobin spectrum of the hemoglobin of an adult disappears rapidly, while that of the hemoglobin of a fetus or a newborn infant persists for some time. The denaturation time of hemoglobin from old stains of blood from a fetus is shorter, however, than that of hemoglobin from fresh stains. It is usually possible to detect the presence or the absence of blood of a fetus or a newborn infant in old blood stains if extracts are prepared and treated with sodium hydroxide. A denaturation time lasting more than one-half hour indicates that the blood stain in question is from a fetus or a newborn infant or an infant less than 5 months of age. A denaturation time lasting from one to five minutes indicates that the blood stain is from an adult or from an infant over 6 months of age. In all such cases the stains are first routinely tested for the presence of human blood, and, if possible, the blood group should also be determined. Control tests with extracts of stains known to represent blood of an adult and a fetus should be made in every case for comparison. Extracts made from blood stains older than several months gave uncertain results.

SUMMARY

The hemoglobin of human fetuses and newborn infants is much more resistant to the denaturizing action of alkali than is the hemoglobin of adults. The denaturation time for the former when treated with 1 per

cent sodium hydroxide is from one to three or more hours; for the latter, from one to five minutes.

The alkali-resistant form of hemoglobin disappears from the blood of infants at about the age of 6 months.

The marked alkali-resistance of the hemoglobin of human fetuses and very young infants interferes with the Hoppe-Seyler alkali test for the detection of carbon monoxide in blood, producing a false positive reaction. This test is useless and misleading when used on the blood of a newborn or a very young infant. The other standard tests for carbon monoxide may be used to detect carbon monoxide in such blood.

The denaturation time of normal fetal blood that has been treated with alkali is even greater than that of adult blood containing carboxyhemoglobin treated in the same way.

In a case of acute carbon monoxide poisoning of a nine months' pregnant woman found dead in a gas-filled room, carbon monoxide was not detectable in the fetal blood although the maternal blood contained 65 per cent carboxyhemoglobin.

Decomposed and dried fetal blood can be differentiated from adult blood by means of the alkali test. Extracts of old blood stains which are brown may be differentiated by spectroscopic examination before and after the addition of alkali. The human character of such stains should be determined first. Control tests should be carried out with known extracts. Very old blood stains give uncertain results.

Notes and News

Awards.—Charles B. Huggins, professor of surgery at the University of Chicago, has been awarded the prize of $2,000 given by Charles L. Mayer and administered by the National Science Fund of the National Academy of Sciences. This prize was offered for the outstanding contribution in 1942 to the knowledge of the growth of animal cells with special reference to cancer. The award to Dr. Huggins was made for his studies of the human prostate and prostatic cancer. A second Charles L. Mayer award will be made for work in 1943. The address of the National Science Fund is 515 Madison Avenue, New York, and nominations for the 1943 prize should be sent in by Jan. 15, 1944. The advisory committee for the fund is interested primarily in fundamental studies of the factors of growth of animal cells.

The American Association of University Women has given its achievement award of $2,500 to Florence Seibert, Phipps Institute, Philadelphia, for her research in tuberculosis.

Appointments.—The Finney-Howell Research Foundation has awarded fellowships for research in cancer as follows, each with an annual stipend of $2,000:

Rose I. Schukoff, of Petrograd, to work at the Glasgow Royal Cancer Hospital (awarded for the third year).

Emilia Vicari, of the Ohio State University, to work at the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine (awarded for the third year).

Borroughs R. Hill, of Tulane University, to work at Harvard University (awarded for the second year).

Nelicia Maier, of Paris, France, to work at Yale University (new).

James A. Miller, of the University of Wisconsin, to work at the University of Wisconsin (new).

Applications for fellowships must be made before Jan. 1, 1944 on blanks furnished by the secretary, 1211 Cathedral Street, Baltimore.

Book Reviews

Atlas of Ovarian Tumors. Gemma Barzilai, M.D., New York. Preface by Fred W. Stewart, M.D., pathologist, Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York. Pp. 264, 8½ by 11, with 258 original illustrations, 45 in colors, on 58 plates. Price $10. New York: Grune & Stratton, 1943.

The illustrations, all of microscopic appearances, and the mechanical features are excellent. The legends describe well the distinctive structural patterns of the tumors; the staining method and the magnification are given in each case. In the text are good systematic accounts of the gross and the microscopic features of the tumors from the point of view of morphology; of the derivation of the tumors; of the nomenclature; of the functional activities, the course and other matters. There is, however, no systematic review of the literature. The lists of the names by which some of these tumors have been known remind the reader of their history. The author's classification is genetic, i. e., based on the cellular derivations of the tumors, and does not take into consideration their cancerous or noncancerous type. Accordingly the primary tumors are grouped as follows:

1. Derived from the follicles—granulosa cell tumor, theca cell tumor.
2. Derived from the male gonad—arrhenoblastoma, virilizing lipoid cell tumor (exact nature not agreed on).
3. Derived from the early mesenchymal core of the ovary—dysgerminoma.
4. Teratoma, embryonal and adult.
5. Fibroma, sarcoma.
6. Derived from structures adjacent to the ovary or, in embryonal stages, closely entwined with the ovarian anlage—Brenner tumor, endosalpingioma, seroanaplastic carcinoma, pseudomucinous adenoma, ovarian and peritoneal pseudomyxoma, pseudomucinous adenocarcinoma, mesonephroma (Schiller).

Endosalpingioma, in group 6, is an ovarian tumor "that duplicates morphologic and functional conditions proper to the epithelium of the normal human tube." Previously this tumor has been called by many names, mostly variations of "cystadenoma" or "adenocystoma." The adjective "seroanaplastic" is applied to ovarian carcinoma in which the epithelial cells do not produce mucus, but why "anaplastic"? Are not all forms of carcinoma more or less anaplastic?

All who are interested in the newer knowledge of the derivation and the structure of ovarian tumors will welcome this atlas. It is a noteworthy accession to the literature in its field.

Medical Jurisprudence and Toxicology. John Glaister, M.D., D.Sc., Fellow of the Royal Faculty of Physicians and Surgeons, Glasgow; barrister-at-law of the Inner Temple, regius professor of forensic medicine, University of Glasgow; formerly professor of forensic medicine, University of Egypt, Cairo, and medicolegal consultant to the Egyptian government. Seventh edition. Pp. 679, with 132 illustrations. Price $8. Baltimore: The Williams & Wilkins Company, 1942.

Once more this book, first published in 1902, has been revised and brought up to date. It is a textbook along traditional lines for students and practitioners, particularly those in England and Scotland. The relevant legal enactments and procedures in those countries are described in necessary detail. The part on toxicology deals with the actions of all kinds of poison, the evidence and the qualitative tests for poisoning and the treatment of poisoning, but the quantitative estimation

of poison is not considered because that is the province of the analyst. The part devoted to medical jurisprudence deals with the following topics: identification; medicolegal aspects of death; death from asphyxia (drowning, suffocation, etc.); death from electricity, lightning and burning; death from criminal neglect and starvation; the medicolegal aspects of wounds; blood stains; sexual medicolegal problems (three chapters); states of insensibility; the medicolegal aspects of lunacy. There are numerous gross illustrations, many of them highly realistic: fingerprints; dead bodies showing putrefactive and other conditions; wounds and injuries of various kinds. The illustrations in color showing a "pugilistic attitude" of a burned human body and poisonous berries and seeds are excellent. Brief instructive accounts are given of illustrative cases. The chapter on blood stains is quite satisfactory. The value of bacteriologic methods in postmortem examinations is not emphasized. The style is direct and effective. The medical student and the practitioner will find this a good practical book in its field.

Books Received

MANUAL OF VETERINARY BACTERIOLOGY. Raymond A. Kelser, D.V.M., Ph.D., brigadier general, United States Army; chief, Veterinary Division, Surgeon General's Office, War Department, Washington, D. C. Harry W. Schoening, V.M.D., chief, Pathological Division, Bureau of Animal Industry, and assistant chief, Bureau of Animal Industry, United States Department of Agriculture, Washington, D. C. Fourth edition. Pp. 719, with 94 figures. Price $6.50. Baltimore: The Williams & Wilkins Company, 1943.

The fourth edition has been revised by Harry W. Schoening, of the United States Department of Agriculture, who has become co-author. The book is more than a manual of veterinary bacteriology because it includes also fungi, protozoa and viruses that cause veterinary infections. The part on pathogenic ultramicroscopic viruses has been rewritten and enlarged to include the recent advances in that important field of veterinary microbiology. The book continues to merit the recommendation in the review of the second edition (ARCH. PATH. **16**:451, 1933) "as a reliable and comprehensive source of information in regard to the micro-organisms of veterinary infections."

VASCULAR SPASM EXPERIMENTAL STUDIES. Alexander John Nedzel, M.D., M.S., associate professor of pathology. Contribution from the Department of Pathology, Bacteriology and Public Health in the University of Illinois College of Medicine. Pp. 151, with 161 figures. Price $2.75 cloth bound and $2.25 paper bound. Urbana, Ill.: University of Illinois Press, 1943.

The monograph presents results of the study of the role of vascular spasm in the genesis of various diseases. Besides reviews of literature, it contains summaries of the author's previous articles and of new experiments. The action of pitressin, which was used for the production of spasm, is discussed; also the role of vascular spasm in the initiation of changes in the valves of the heart leading to endocarditis. The production by pitressin of gastric ulcers and of hepatic and renal lesions is considered. The alterations produced in the central nervous system are interpreted as analogous to those of multiple sclerosis in man.

THE INNER EAR, INCLUDING OTONEUROLOGY, OTOSURGERY AND PROBLEMS IN MODERN WARFARE. Joseph Fischer, M.D., staff member, Beth Israel Hospital, Boston; formerly associate of Adam Politzer and senior otolaryngologist with Gustav Alexander of the Policlinic of Vienna. Louis E. Wolfson, M.D., instructor in ear, nose and throat, Tufts Medical School; senior surgeon in ear, nose and throat, Boston Dispensary. Pp. xii and 421, with 76 figures. Price $5.75. New York: Grune & Stratton, 1943.

The contents are: clinical anatomy; general physiology; applied physiology; functional tests; primary diseases of the labyrinthine capsule: otosclerosis; inflammatory diseases of the inner ear; intracranial labyrinthogenic complications; chemotherapy; facial palsy; congenital diseases; neoplasms; vascular lesions: Ménière's syndrome; war trauma; the role of the inner ear in aeronautics; effects of atmospheric pressure changes on the ear.

BRUCELLOSIS IN MAN AND ANIMALS. I. Forest Huddleson, D.V.M., M.S., Ph.D., research professor in bacteriology, Michigan State College. Contributing authors: A. V. Hardy, M.D., Dr.P.H., associate professor of epidemiology, DeLamar Institute of Public Health, Columbia University Medical School; consultant, United States Public Health Service. J. E. Debono, M.D., M.R.C.P., professor of pharmacology and therapeutics, Royal University of Malta. Ward Giltner, D.V.M., M.S., Dr. P. H., dean of veterinary division and professor of bacteriology, Michigan State College. Revised edition. Pp. xv and 379, with 42 figures. Price $3.50. New York: The Commonwealth Fund, 1943.

The contents are: the genus Brucella; methods of isolating Brucella; differentiation of the species of the genus Brucella; brucellosis in human beings; brucellosis in animals; laboratory diagnosis of brucellosis; eradication or control of sources of Brucella infection.

BLOOD GROUPS AND TRANSFUSION. Alexander S. Wiener, M.D., serologist and bacteriologist in the Office of the Chief Medical Examiner of New York City; head of transfusion division, Jewish Hospital of Brooklyn. Pp. 438, with 69 figures. Price $7.50. Springfield, Ill.: Charles C Thomas, Publisher, 1943.

There are two new chapters, one dealing with the transfusion of stored blood and blood substitutes, the other with the Rh factor and its role in fetal erythroblastosis. The old chapters have been extensively revised. The book presents well the present knowledge of the theory and practice of blood grouping as well as that of transfusion.